

22nd Century Limited, LLC

June 21, 2011

Bob Rappaport, M.D., Director
Division of Anesthesia and Analgesia Products
Center for Drug Evaluation and Research
Food and Drug Administration
5901-B Ammendale Road
Beltsville, MD 20705-1266

RE: IND 103,589 (SN000) X-22 Smoking Cessation Product
Initial Investigational New Drug Application
Request for Fast Track Designation

Dear Dr. Rappaport:

22nd Century Limited, LLC (22nd Century) is herewith submitting the Initial Investigational New Drug application for X-22 Smoking Cessation Product for the indication of smoking cessation. X-22 Smoking Cessation Product is a tobacco-based botanical drug product containing proprietary very low nicotine (VLN) cigarettes that could be of use in individuals trying to quit smoking.

22nd Century is a plant biotechnology company focused on the discovery, research and clinical development of products for the treatment of smoking. 22nd Century was formed in 1998 to support research and development on nicotine biosynthesis in plants with researchers who were affiliated with North Carolina State University. The head office of 22nd Century is located in Williamsville, New York.

22nd Century believes that the scientific rationale for and clinical experience with a similar product also containing 22nd Century's licensed VLN tobacco indicates that X-22 Smoking Cessation Product could be a useful addition or replacement to the available therapies for smoking cessation and should be studied in a systematic manner under an IND.

This IND submission details the manufacturing processes for X-22 Smoking Cessation Product and the clinical and nonclinical safety and previous human experience with the same VLN tobacco as used in X-22 cigarettes. The first study to be conducted under this IND is a double-blind, randomized, active-controlled study in subjects who have expressed a desire to quit smoking. This trial (X-22-201) is designed to assess the safety and efficacy of X-22. In the initial IND study, approximately 216 subjects, including both males and females, will receive X-22 cigarettes or an active control cigarette daily for 6 weeks.

Reference is made to pre-IND submissions for PIND 103,589, made to FDA on December 27, 2008, and to our Type B, pre-IND meeting with members of the Office of Nonprescription Products, the Division of Anesthesia, Analgesia and Rheumatology Products, the Office of New Drug Quality Assessment, the Division of Nonprescription Clinical Evaluation, the Office of

Clinical Pharmacology, and the Botanical Review Team of the Office of Drug Evaluation 1 on January 21, 2009, to discuss the Initial IND submission package and development program for X-22 Smoking Cessation Product.

We have prepared the Initial IND document in a manner to address FDA's comments and requests received at the pre-IND meeting and in FDA's letter of February 19, 2009 which included the official minutes of the pre-IND meeting. A response document that specifically addresses the comments and requests is attached to Items 3 and 4 in Volume 1 of the IND. Where appropriate, information in response to FDA's comments and requests has also been incorporated into the body of the IND.

Reference is also made to my telephone conversation on June 14, 2011, with Ms. Parinda Jani, Project Manager in your Division, regarding the number of copies of the IND required for review. As instructed by Ms. Jani, we are submitting three complete copies of the IND (Volumes 1 through 12) to the Central Document Room for the IND file. We are also submitting a desk copy of Volume 1 of the IND. In addition, we are providing electronic (PDF) copies of the main IND documents on a CD for the use of the reviewing staff. The desk copy of the Volume 1 and the CD will be sent directly to Ms. Jani

We are also submitting within this IND, as Item 10, Additional Information, a request for Fast Track Designation for X-22 Smoking Cessation Product. It has been well established that cigarette smoking is toxic and poses serious health risks to subjects, namely cardiovascular disease, multiple cancers, pulmonary disease, adverse reproductive outcomes, and exacerbation of other chronic health conditions, and that current smoking cessation therapies are effective in a minority of smokers. 22nd Century believes that X-22 Smoking Cessation Product has the potential, via smoking cessation, to prevent a host of serious medical conditions and meet this critical medical need. Background information regarding smoking-related health problems and smoking cessation is provided in Item 10 of this IND, as referenced above. References cited in the request are attached to this submission or are available on the web site indicated in the citation.

We appreciate the time and effort to be expended by the Division during the review of this IND submission for X-22 Smoking Cessation Product and our request for Fast Track Designation and we look forward to working with the Division during the review process. If you have additional questions or require further information, please do not hesitate to contact me by e-mail at (b) (4), (b) (6) or by telephone at (b) (4), (b) (6).

Sincerely,

(b) (4), (b) (6)

22nd Century Limited, LLC
Regulatory Affairs Consultant

cc: J. Pandolfino
M. Moynihan

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

INVESTIGATIONAL NEW DRUG APPLICATION (IND)
(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)

Form Approved: OMB No. 0910-0014.
Expiration Date: May 31, 2009
See OMB Statement on Reverse.

NOTE: No drug may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40).

1. NAME OF SPONSOR
22nd Century Limited, LLC

2. DATE OF SUBMISSION
06/21/2011

3. ADDRESS *(Number, Street, City, State and Zip Code)*
8201 Main Street
Suite 6
Williamsville, New York 14221

4. TELEPHONE NUMBER *(Include Area Code)*
(b) (4), (b) (6)

5. NAME(S) OF DRUG *(Include all available names: Trade, Generic, Chemical, Code)*
X-22 Smoking Cessation Product

6. IND NUMBER *(If previously assigned)*
103589

7. INDICATION(S) *(Covered by this submission)*
Smoking cessation

8. PHASE(S) OF CLINICAL INVESTIGATION TO BE CONDUCTED:
☐ PHASE 1 ☑ PHASE 2 ☐ PHASE 3 ☐ OTHER _____ *(Specify)*

9. LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS (21 CFR Part 312), NEW DRUG OR ANTIBIOTIC APPLICATIONS *(21 CFR Part 314)*, DRUG MASTER FILES *(21 CFR Part 314.420)*, AND PRODUCT LICENSE APPLICATIONS (21 CFR Part 601) REFERRED TO IN THIS APPLICATION.
IND # 69,185 (Letter of cross-reference authorization attached)

10. ***IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 0000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 0001." Subsequent submissions should be numbered consecutively in the order in which they are submitted.***

SERIAL NUMBER
0

11. THIS SUBMISSION CONTAINS THE FOLLOWING: *(Check all that apply)*
☑ INITIAL INVESTIGATIONAL NEW DRUG APPLICATION (IND)
☐ RESPONSE TO CLINICAL HOLD

PROTOCOL AMENDMENT(S):
☐ NEW PROTOCOL
☐ CHANGE IN PROTOCOL
☐ NEW INVESTIGATOR

INFORMATION AMENDMENT(S):
☐ CHEMISTRY/MICROBIOLOGY
☐ PHARMACOLOGY/TOXICOLOGY
☐ CLINICAL

IND SAFETY REPORT(S):
☐ INITIAL WRITTEN REPORT
☐ FOLLOW-UP TO A WRITTEN REPORT

☐ RESPONSE TO FDA REQUEST FOR INFORMATION
☐ ANNUAL REPORT
☐ GENERAL CORRESPONDENCE
☐ REQUEST FOR REINSTATEMENT OF IND THAT IS WITHDRAWN, INACTIVATED, TERMINATED OR DISCONTINUED
☑ OTHER Request for Fast Track Designation *(Specify)*

CHECK ONLY IF APPLICABLE

JUSTIFICATION STATEMENT MUST BE SUBMITTED WITH APPLICATION FOR ANY CHECKED BELOW. REFER TO THE CITED CFR SECTION FOR FURTHER INFORMATION.

☐ **TREATMENT IND 21 CFR 312.35(b)** ☐ **TREATMENT PROTOCOL 21 CFR 312.35(a)** ☐ **CHARGE REQUEST/NOTIFICATION 21 CFR312.7(d)**

FOR FDA USE ONLY

CDR/DBIND/DGD RECEIPT STAMP	DDR RECEIPT STAMP	DIVISION ASSIGNMENT:
		IND NUMBER ASSIGNED:

12. **CONTENTS OF APPLICATION**

This application contains the following items: *(Check all that apply)*

- ☑ 1. Form FDA 1571 *[21 CFR 312.23(a)(1)]*
- ☑ 2. Table of Contents *[21 CFR 312.23(a)(2)]*
- ☑ 3. Introductory statement *[21 CFR 312.23(a)(3)]*
- ☑ 4. General Investigational plan *[21 CFR 312.23(a)(3)]*
- ☑ 5. Investigator's brochure *[21 CFR 312.23(a)(5)]*
- ☑ 6. Protocol(s) *[21 CFR 312.23(a)(6)]*
  - ☑ a. Study protocol(s) *[21 CFR 312.23(a)(6)]*
  - ☑ b. Investigator data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - ☑ c. Facilities data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - ☑ d. Institutional Review Board data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
- ☑ 7. Chemistry, manufacturing, and control data *[21 CFR 312.23(a)(7)]*
  - ☑ Environmental assessment or claim for exclusion *[21 CFR 312.23(a)(7)(iv)(e)]*
- ☑ 8. Pharmacology and toxicology data *[21 CFR 312.23(a)(8)]*
- ☑ 9. Previous human experience *[21 CFR 312.23(a)(9)]*
- ☑ 10. Additional information *[21 CFR 312.23(a)(10)]*

13. IS ANY PART OF THE CLINICAL STUDY TO BE CONDUCTED BY A CONTRACT RESEARCH ORGANIZATION? ☑ YES ☐ NO

IF YES, WILL ANY SPONSOR OBLIGATIONS BE TRANSFERRED TO THE CONTRACT RESEARCH ORGANIZATION? ☑ YES ☐ NO

IF YES, ATTACH A STATEMENT CONTAINING THE NAME AND ADDRESS OF THE CONTRACT RESEARCH ORGANIZATION, IDENTIFICATION OF THE CLINICAL STUDY, AND A LISTING OF THE OBLIGATIONS TRANSFERRED.

14. NAME AND TITLE OF THE PERSON RESPONSIBLE FOR MONITORING THE CONDUCT AND PROGRESS OF THE CLINICAL INVESTIGATIONS

Michael R. Moynihan, Ph.D.

VP Research and Development, 22nd Century Limited, LLC

15. NAME(S) AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW AND EVALUATION OF INFORMATION RELEVANT TO THE SAFETY OF THE DRUG

(b) (4), (b) (6) Medical Monitor

(b) (4)

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set fourth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

16. NAME OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE

(b) (4)
Regulatory Affairs Consultant for 22nd Century

17. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED

(b) (6), (b) (4)

18. ADDRESS *(Number, Street, City, State and Zip Code)*

(b) (4)

19. TELEPHONE NUMBER *(Include Area Code)*

(b) (4), (b) (6)

20. DATE

06/21/2011

(**WARNING**: A willfully false statement is a criminal offense. U.S.C. Title 18, Sec. 1001.)

Public reporting burden for this collection of information is estimated to average 100 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research (HFM-99)
1401 Rockville Pike
Rockville, MD 20852-1448

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Please DO NOT RETURN this application to this address.

Vector Tobacco Inc.

Marc N. Bell
Senior Vice President & General Counsel

Tel (b) (4), (b) (6)
Fax
(b) (6)

January 30, 2009

VIA FEDERAL EXPRESS
Food and Drug Administration
Attention: Charles J. Ganley, MD
Director of OTC Drug Products
Center for Drug Evaluation and Research
Document Control Room
5901 - B Ammendale Rd.
Beltsville, MD 20705-1266

RE: Limited Right of Reference

Dear Dr. Ganley:

Vector Tobacco Inc. ("Sponsor") hereby grants a limited right of reference under 21 C.F.R. § 312.23(b) to 22nd Century Limited, LLC ("22nd Century") for IND 69,185. Pursuant to the Opinion and Final Award of Arbitrator, issued on February 21, 2008, 22nd Century is granted a limited right of reference to selected portions of IND 69,185. 22nd Century is not granted a right of reference to certain "exempt information" and information, submissions, responses, questions or amendments related to IND 69,185 dated after December 31, 2006.

Sponsor has enclosed three (3) copies of the redacted IND containing selected portions of IND 69,185 that 22nd Century may reference pursuant to this limited right of reference. In addition to the redacted material enclosed herewith, 22nd Century is not entitled to reference anything dated after December 31, 2006. The enclosed redacted IND file contains the **only** information 22nd Century is entitled to reference pursuant to this limited right of reference. This limited right of reference is restricted to 22nd Century and may not be assigned, sold, licensed, sub-licensed, or transferred in any manner to any other party, unless approved in writing by Sponsor.

Very truly yours,

(b) (4), (b) (6)

MARC N. BELL

Enclosures

Item 13. List of Sponsor Obligations Transferred

For Clinical Protocol No. X-22-201 " A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid" the following obligations are transferred:

Sponsor Obligations Transferred	**Group and Contact Person Assuming Obligations**
Medical monitoring of subjects Review of safety information	(b) (6), (b) (4)
Regulatory contact Notification of investigators for SAEs Notification of FDA for SAEs	
Investigator and site selection Obtain information from investigators	
Clinical trial material distribution to the investigational sites	
Control of investigational drug shipment	
Investigational drug recordkeeping	
Monitor selection	

Study monitoring	(b) (6), (b) (4)	
Study recordkeeping		

See OMB Statement on Reverse. Form Approved: OMB No. 0910-0616, Expiration Date: 06-30-2008

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. NAME OF SPONSOR/APPLICANT/SUBMITTER
   22nd Century Limited, LLC

2. DATE OF THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES
   06/21/2011

3. ADDRESS *(Number, Street, State, and ZIP Code)*
   8201 Main Street
   Suite 6
   Williamsville, NY 14221

4. TELEPHONE AND FAX NUMBER *(Include Area Code)*
   (Tel.) (b) (6)
   (Fax)

PRODUCT INFORMATION

5. **FOR DRUGS/BIOLOGICS:** Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s)
   FOR DEVICES: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)
   (Attach extra pages as necessary)

   X-22 Smoking Cessation Product

APPLICATION / SUBMISSION INFORMATION

6. TYPE OF APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

   ☒ IND ☐ NDA ☐ ANDA ☐ BLA ☐ PMA ☐ HDE ☐ 510(k) ☐ PDP ☐ Other

7. INCLUDE IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/OTHER NUMBER *(If number previously assigned)*
   103,589

8. SERIAL NUMBER ASSIGNED TO APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES
   000

CERTIFICATION STATEMENT / INFORMATION

9. CHECK ONLY ONE OF THE FOLLOWING BOXES *(See instructions for additional information and explanation)*

   ☐ A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

   ☐ B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

   ☒ C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

10. IF YOU CHECKED BOX C, IN NUMBER 9, PROVIDE THE NATIONAL CLINICAL TRIAL (NCT) NUMBER(S) FOR ANY "APPLICABLE CLINICAL TRIAL(S)," UNDER 42 U.S.C. § 282(j)(1)(A)(i), SECTION 402(j)(1)(A)(i) OF THE PUBLIC HEALTH SERVICE ACT, REFERENCED IN THE APPLICATION/ SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES *(Attach extra pages as necessary)*
    NCT Number(s): In Progress

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act.
Warning: A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

11. SIGNATURE OF SPONSOR/APPLICANT/SUBMITTER OR AN AUTHORIZED REPRESENTATIVE

(b) (4), (b) (6)

12. NAME AND TITLE OF THE PERON WHO SIGNED IN NO. 11
    [redacted] (b) (4), (b) (6)
    (Title) Regulatory Affairs Consultant for 22nd Century

13. ADDRESS *(Number, Street, State, and ZIP Code) (of person identified in No. 11 and 12)*

(b) (4), (b) (6)

14. TELEPHONE AND FAX NUMBER *(Include Area Code)*
    (Tel.) (b) (4), (b) (6)
    (Fax)

15. DATE OF CERTIFICATION
    06/21/2011

PSC Graphics: (301) 443-1090 EF

Instructions for Completion of Form FDA 3674

Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))
Form 3674 must accompany an application/submission, including amendments, supplements, and resubmissions, submitted under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.

1. **Name of Sponsor/Applicant/Submitter -** This is the name of the sponsor/applicant/submitter of the drug/biologic/device application/ submission which the certification accompanies. The name must be identical to that listed on the application/submission.

2. **Date -** This is the date of the application/submission which the certification accompanies.

3. **& 4. -** Provide complete address, telephone number and fax number of the sponsor/applicant/submitter.

5. **Product Information - For Drugs/Biologics:** Provide the established, proprietary name, and/or chemical/biochemical/blood product/ cellular/gene therapy name(s) for the product covered by the application/submission. Include all available names by which the product is known. **For Devices:** Provide the common or usual name, classification, trade or proprietary or model name(s), and/or model number(s). Include all available names/model numbers by which the product is known.

6. **Type of Application/Submission -** Identify the type of application/submission which the certification accompanies by checking the appropriate box. If the name of the type of application/submission is not identified, check the box labeled "Other."

7. **IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/Other Number -** If FDA has previously assigned a number associated with the application/ submission which this certification accompanies, list that number in this field. For example, if the application/submission accompanied by this certification is an IND protocol amendment and the IND number has already been issued by FDA, that number should be provided in this field.

8. **Serial Number -** In some instances a sequential serial number is assigned to the application. If there is such a serial number, provide it in this field.

9. **Certification -** This section contains three different check-off boxes.
   Box A should be checked if the sponsor/applicant/submitter has concluded that the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, do not apply because no clinical trials are included, relied upon, or otherwise referred to, in the application/ submission which the certification accompanies.

   Box B should be checked if the sponsor/applicant/submitter has concluded that the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, do not apply at the time of submission to any clinical trials that are included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies. This means that, at the time the application/submission is being made, the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, do not apply to any of the clinical trials included, relied upon, or otherwise referred to, in the application/submission which this certification accompanies.

   Box C should be checked if the sponsor/applicant/submitter has concluded that the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, do apply at the time of submission to some or all of the clinical trials that are included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies. This means that, at the time the application/ submission is being made, the requirements of 42 U.S.C. § 282(j), section 402(j) of the Public Health Service Act, apply to one or more of the clinical trials included, relied upon, or otherwise referred to, in the application/submission which this certification accompanies.

10. **National Clinical Trial (NCT) Numbers -** If you have checked Box C in number 9 (Certification), provide the NCT Number obtained from www.ClinicalTrials.gov for each clinical trial that is an "applicable clinical trial" under 42 U.S.C. § 282(j)(1)(A)(i), section 402(j)(1)(A)(i) of the Public Health Service Act, and that is included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies. Type only the number, as NCT will be added automatically before number. Include any and all NCT numbers assigned to the clinical trials included, relied upon, or otherwise referred to, in the application/submission which this certification accompanies. Multiple NCT numbers may be required for a particular certification, depending on the number of "applicable clinical trials" included, relied upon, or otherwise referred to, in the application/submission which the certification accompanies.

11. **Signature of Sponsor/Applicant/Submitter or an Authorized Representative -** The person signing the certification must sign in this field.

12. **Name and Title of Person Who Signed in number 11. -** Include the name and title of the person who is signing the certification. If the person signing the certification is not the sponsor/applicant/submitter of the application/submission, he or she must be an authorized representative of the sponsor/applicant/submitter.

13. **& 14. -** Provide the full address, telephone and fax number of the person who is identified in number 11 and signs the certification in number 11.

15. Provide the date the certification is signed. This date may be different from the date provided in number 2.

Paperwork Reduction Act Statement

Public reporting burden for this collection of information is estimated to average 15 minutes and 45 minutes (depending on the type of application/submission) per response, including time for reviewing instructions. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to the applicable address below.

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
Form No. FDA 3674
5901-B Ammendale Road
Beltsville, MD 20705-1266

Food and Drug Administration
Center for Biologics Evaluation and Research
1401 Rockville Pike
Rockville, MD 20852-1448

Food and Drug Administration
Center for Devices and Radiological Health
Program Operations Staff (HFZ-403)
9200 Corporate Blvd.
Rockville, MD 20850

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information, unless it displays a currently valid OMB control number.

FDA-3674 (1/08) (BACK)

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	Laurence Chu, M.D. – 1572, CV, and Medical License	1	
	Robert J. Jeanfreau, M.D. – 1572, CV, and Medical License	1	
	Ivan H. Rarick II, M.D. – 1572, CV, and Medical License	1	
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ITEMS 3 AND 4
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3. INTRODUCTORY STATEMENT AND GENERAL INVESTIGATIONAL PLAN [21 CFR 312.23(A)(3)]

3.1. Introductory Statement

22nd Century Limited, LLC (22nd Century) intends to develop its investigational product, X-22 Smoking Cessation Product, for the indication of smoking cessation. The product is intended to reduce cravings for cigarettes in smokers and increase the chances of successfully quitting smoking.

X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product manufactured using very low nicotine (VLN) processed tobacco from the very low alkaloid variety tobacco plant, Vector 21-41, as the active ingredient. Consequently, the X-22 cigarette is a VLN cigarette. Like conventional cigarettes, X-22 cigarettes consist of filler, filter, and wrapper components. X-22 cigarettes contain menthol, (b) (4) The filler in X-22 cigarettes, like that in conventional cigarettes, is comprised primarily of cured tobacco leaf and does not contain non-tobacco plant material (other than a relatively small amount present in some of the additives and flavorings included in the filler). Other inactive ingredients are the filter and wrapper components. All processing of tobacco is carried out by processors of conventional tobacco. All manufacturing of cigarette components and cigarettes is carried out by manufacturers of conventional cigarette components and cigarettes.

The X-22 cigarette is administered by combustion and inhalation of its smoke. X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of less than 1 mg/g and a machine smoking yield of ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes or the University of Kentucky Reference Reference (KY2R4F or KY3R4F) cigarettes (the tobacco industry analytical reference standard). The composition of smoke from the X-22 cigarette is chemically similar to the composition of smoke from conventional cigarettes. Like conventional cigarettes, X-22 cigarettes are toxic and pose health risks to subjects, namely cancer, cardiovascular disease, respiratory disease and reproductive effects.

VLN tobacco from the tobacco variety Vector 21-41 was also used (under license from 22nd Century) to produce Quest 3® brand cigarettes (Vector Tobacco, Inc.) and was described in their IND 69,185 filings, to which 22nd Century has right of reference to their non-redacted IND documents.

22nd Century has previously met with members of the Office of Nonprescription Products, the Division of Anesthesia, Analgesia and Rheumatology Products, the Office of New Drug Quality Assessment, the Division of Nonprescription Clinical Evaluation, the Office of Clinical Pharmacology, and the Botanical Review Team of the Office of Drug Evaluation 1 in a Pre-IND meeting on January 21, 2009, to discuss the Initial IND submission package and development program for X-22 Smoking Cessation Product. Minutes of this meeting are attached in **Appendix 1** of this document. As a result of this meeting, 22nd Century has

decided to seek development of X-22 Smoking Cessation Product as an investigational prescription drug product under the review of the Division of Anesthesia and Analgesia Products for the indication of smoking cessation.

We have prepared the Initial IND document in a manner to address FDA's comments and requests received at the pre-IND meeting. We are providing a response to FDA comments on the development of X-22 Smoking Cessation Product, as summarized in the meeting minutes, as **Appendix 2** of this document. Where appropriate, information in response to FDA's comments and requests has also been incorporated into the body of the IND. These locations are noted in the response document.

3.2. Name of Drug, Active Ingredient, Formulation, Route of Administration

3.2.1. X-22 Smoking Cessation Product

X-22 Smoking Cessation Product is a tobacco-based cigarette. It is a botanical drug product manufactured using VLN processed tobacco from the very low alkaloid variety tobacco plant Vector 21-41 as the active ingredient. Consequently, the X-22 cigarette is a VLN cigarette. Like conventional cigarettes, X-22 cigarettes consist of filler, filter, and wrapper components. X-22 cigarettes contain menthol, (b) (4) The filler in X-22 cigarettes, like that in conventional cigarettes, is comprised primarily of cured tobacco leaf and does not contain non-tobacco plant material (other than a relatively small amount present in some of the additives and flavorings included in the filler). Other inactive ingredients are the filter and wrapper components, which are supplied by providers of components for conventional cigarettes. The X-22 cigarette is administered by combustion and inhalation of its smoke.

Active Ingredient:	X-22 processed VLN tobacco
Inactive Ingredients:	casing, filter, wrapper, adhesives, flavorings (including menthol, (b) (4))
Formulation(s):	X-22 cigarettes will be packaged in a standard cigarette configuration with a filter intended for oral inhalation of the smoke.
Route of Administration:	Combustion for Oral Inhalation of the Smoke.

3.3. Summary of Previous Human Experience

3.3.1. X-22 Smoking Cessation Product

X-22 Smoking Cessation Product has not previously been administered to humans.

3.3.2. Similar Smoking Cessation Products

The same VLN tobacco variety used in X-22 cigarettes, Vector 21-41, was also used in Quest® and Quest menthol cigarettes. Quest cigarettes were sold in the U.S. by Vector Tobacco Inc. from December 2002 to December 2007, and Quest menthol cigarettes were sold by Vector Tobacco Inc. from November 2003 through December 2007. Quest remained

in the tobacco trade pipeline, including sales by retailers, through 2009. According to filings by Vector Group Ltd. with the Securities and Exchange Commission, sales of Quest were approximately $60 million during this timeframe. This equates to approximately 26 million packs or 520 million cigarettes sold that contained Vector 21-41. In other words, this equates to 14,246 pack-a-day smokers for 5 years.

Quest yielded 8 to 10 mg "tar" and was available in 3 varieties: a VLN cigarette, Quest 3® (nicotine yield ≤0.05 mg) and two low nicotine cigarettes Quest 2® (nicotine yield 0.3 mg) and Quest 1® (nicotine yield 0.6 mg). Vector 21-41 was blended with typical tobacco (containing approximately 2% nicotine) in Quest 1 and Quest 2 cigarettes. Quest 1 contains slightly less nicotine than an average "light" cigarette and Quest 2 contains half the nicotine content of Quest 1 and approximately 6 times the nicotine content of Quest 3.

The X-22 cigarette is most similar to Quest 3, a VLN cigarette that yielded ≤0.05 mg nicotine per cigarette. Vector 21-41 was the only tobacco in Quest 3 and is also the only tobacco in the X-22 cigarette.

Quest 3 cigarettes have also been used in various studies, including clinical trials evaluating their usefulness as an aid to smoking cessation. In many cases, researchers purchased Quest at retail for their studies. Smoking cessation trials utilizing Quest 3 are described in Item 9 Previous Human Experience (See Section 2.1) of this IND.

VLN cigarettes containing Vector 21-41 and designed by 22nd Century are being used in a Phase II clinical trial currently in progress, "Innovative Interventions for Smoking Cessation" (ClinicalTrials.gov Identifier: NCT01050569). This trial is evaluating the effect of a combination of a 21 mg nicotine patch and VLN cigarette compared to the VLN cigarette alone or 21 mg nicotine patch alone on abstinence and withdrawal. (It should be noted that Quest 3 is the cigarette listed as being used in the description of this clinical trial, but that is not the case.)

This study is described in further detail in the Item 9 (See Section 2.1).

3.4. Withdrawal from Investigation or Marketing

X-22 Smoking Cessation Product has not been withdrawn from investigation or marketing in any country for any reason.

4. BRIEF DESCRIPTION OF THE OVERALL PLAN FOR INVESTIGATING THE DRUG FOR THE FOLLOWING YEAR

4.1 Rationale for the Development of X-22 Smoking Cessation Product

It has been well established that cigarette smoking is toxic and poses health risks to subjects, namely cancer, cardiovascular disease, respiratory disease, and reproductive effects. Cigarette smoking is a complex behavior that is sustained by the pharmacological properties of nicotine and reinforcing behavioral and sensory cues. Currently available pharmacological approaches for smoking cessation in combination with counseling and education (maximal intervention) are effective in a minority of smokers, with the probability of initial success with all types of nicotine replacement therapy (NRT) increased 1.5 to 2.5 fold compared to placebo. Relapse is common following NRT use, and 75 to 80 percent of smokers who receive treatment are unable to achieve long-term abstinence at 6 or 12 months. Therefore, there is an important public health need to provide additional smoking cessation treatments, reduce relapse, and to provide alternative treatments for smokers who may not succeed with, or do not have access to, treatment that includes approved pharmacotherapies.

VLN cigarettes consistently reduce craving for cigarettes and withdrawal in smokers. In contrast to conventional low-yield ("light" or "ultra light") cigarettes that have reduced tar and nicotine yields mainly due to smoke dilution, VLN cigarettes deliver greatly reduced ratios of nicotine to other smoke components. The data from studies of the behavioral and pharmacodynamic properties of VLN cigarettes indicate that a product that delivers the sensory and behavioral cues associated with cigarette use but does not deliver a satisfying amount of nicotine breaks the association of those cues with the contingent delivery of nicotine reinforcement and promotes extinction of smoke cues.

Previous clinical experience with VLN cigarettes is described in further detail in the Item 9 summary section (See Section 2.1 of Item 9).

4.2 Indications to be Studied in the First Year

X-22 Smoking Cessation Product is being investigated for the indication of smoking cessation.

4.3 General Approach to Clinical Investigations

The goal of the Phase II development program will be to evaluate the safety and efficacy profile of X-22 Smoking Cessation Product, the pharmacokinetics of nicotine in the X-22 cigarette, and to determine the optimum dosing regimen in order to design the pivotal Phase III clinical trials for the desired indication.

We will conduct studies to evaluate the single and multiple dose pharmacokinetics of nicotine in the X-22 cigarette and the relative bioavailability of nicotine from X-22 cigarettes compare to other sources of NRT, prior to the commencement of Phase III studies.

Phase III studies will be conducted to further evaluate the safety and efficacy of X-22 cigarettes as an aid to smoking cessation. Any dose response with X-22 cigarettes will be evaluated in Phase II b and/or Phase III studies.

4.4 Clinical Trials to be Conducted During the First Year

4.4.1 X-22-201

The first trial to be conducted under this IND is X-22-201 "A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid".

This study will evaluate the safety and efficacy of X-22 as a smoking cessation aid. The design of X-22-201 is summarized below. The full protocol for this trial is included in this IND application in Item 6.

Title of the Trial

A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid

Clinical Phase

Phase II safety and efficacy trial.

Trial Design

This will be a prospective, randomized, multicenter, double blind, parallel, active controlled Phase II clinical trial in which a total of 216 healthy smokers will be enrolled and followed over 5 months at a maximum of 3 sites.

Trial Objectives

Primary:

- To evaluate the efficacy of X-22 as a smoking cessation aid.

Secondary:

- To assess the effect of X-22 on abstinence at 2 and 3 months after the quit date,
- To assess withdrawal symptoms,
- To evaluate the effect of X-22 on cigarette consumption prior to the quit date
- To evaluate the tolerability of X-22 as a smoking cessation aid.

Exploratory:

- To evaluate other factors that may have an influence on the efficacy of X-22 as a smoking cessation aid, including subject gender, results of The Sensory Questionnaire prior to the quit date, and results of the Fagerström Test of Nicotine Dependence prior to the quit date.

- To compare nicotine use between groups over time.

Criteria for Evaluation:

Safety

All AEs spontaneously reported, elicited, or observed by the investigators will be recorded at each study visit. Heart rate, blood pressure, and body weight will be measured at each study visit. Physical examinations and urine pregnancy tests will be conducted at Screening and Visit 7. Clinical laboratory tests will be conducted at Screening and Visit 5.

Efficacy

The primary efficacy measurement is the number of subjects with 4 weeks of continuous abstinence from smoking cigarettes (Visits 5 to 7) as assessed by subject diary and confirmed by exhaled CO.

Secondary efficacy measurements include smoking abstinence during the follow-up period; withdrawal symptoms as measured by the Minnesota Nicotine Withdrawal Scale at each study visit; the degree of dependence on smoking as measured at Screening and during the treatment phase using the Fagerström Test of Nicotine Dependence; results of a sensory questionnaire, which will be administered to obtain detailed information about the sensory properties of the X-22 cigarettes; and cotinine levels measured at each study visit to estimate nicotine use over time.

4.5 Estimated Number of Subjects to Receive X-22 Smoking Cessation Product during the First Year

Up to 216 male and female adults are planned to be given X-22 cigarettes during the first year of the IND.

4.5.1 Risk Considerations Based on Nonclinical and Clinical Data

4.5.1.1 Nonclinical Data

The nonclinical safety data and experience with VLN cigarettes suggest that VLN cigarettes (and, by extension, X-22 cigarettes) manufactured with Vector 21-41 tobacco pose no new hazard or health risk beyond the risks known for cigarettes manufactured from traditional, non-transgenic tobacco and that, under the conditions of usage proposed in the clinical trial, X-22 cigarettes should not pose any additional risk to subjects in the study beyond those already know to be attributable to smoking standard cigarettes.

4.5.1.2 Clinical Data

The use of X-22 cigarettes in human subjects in the proposed clinical trial is supported by the clinical data described in this IND in the following ways:

- Short term use of ≤0.05 mg nicotine cigarettes for 2 weeks is less effective than longer term use over 6 weeks.
- Smoking ≤0.05 mg nicotine cigarettes for at least 6 weeks alone also seems to be more effective that using a step-down approach (e.g., 2 weeks of 0.6 mg nicotine cigarettes, followed by 2 weeks of 0.3 mg nicotine cigarettes, followed by 2 weeks of ≤0.05 mg nicotine cigarettes).
- Previous clinical studies have found that, when smoking VLN cigarettes, smokers generally consume fewer cigarettes per day than when they smoke their usual brand.
- Previous studies have found no clinical evidence that VLN cigarettes are any less safe than regular cigarettes.
- VLN cigarettes (and, by extension, X-22 cigarettes) manufactured with Vector 21-41 tobacco pose no new hazard or health risk beyond the risks known for traditional, non-transgenic tobacco and, under the conditions of usage proposed in the clinical trial, X-22 cigarettes should not pose any additional risk to subjects in the study beyond those already know to be attributable to smoking standard cigarettes.

4.5.1.3 Overall Cigarette smoking is toxic and poses health risks to subjects, namely cancer, cardiovascular disease, respiratory disease and reproductive effects. The benefits of a reduction in the burden of disease attributable to smoking are desirable, however smoking is a complex behavior and currently available pharmacological approaches for smoking cessation in combination with counseling and education (maximal intervention) are effective in only a minority of smokers. Therefore, there is an important public health need to provide additional smoking cessation treatments, reduce relapse, and to provide alternative treatments for smokers who may not succeed with, or do not have access to, treatment that includes currently approved pharmacotherapies.

There is no information from the nonclinical and clinical data reviewed for this IND that would preclude the daily administration of X-22 cigarettes to male and female subjects in the proposed Phase II clinical trial, X-22-201. Previous clinical studies, as described in Item 9, have shown that the use of VLN cigarettes is associated with consistently reduced craving for cigarettes, reduced withdrawal symptoms, and increase in smoking cessation compared to the use of NRT. This proposed Phase II study will assess the magnitude of effectiveness that can be obtained with the X-22 VLN cigarette as a smoking cessation aid. The results of this study will serve to optimize the design of the pivotal trials (Phase III), and provide objective data to support a decision regarding the development of the product concepts.

It is the goal of the US Department of Health and Human Services, as outlined in the program *Healthy People 2010*, to reduce cigarette smoking in the United States to 12% by the year 2020. 22nd Century believes that the X-22 Smoking Cessation Product, based on the scientific rationale and clinical and nonclinical data provided in this IND, could be an important tool in reaching that goal and should be studied in a systematic manner under an IND.

APPENDIX 1

MINUTES OF JANUARY 21, 2009 PRE-IND MEETING

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

PIND 103,589

XX 22nd Century Limited, LLC
Attention: Michael R. Moynihan, Vice President
Research & Development
8201 Main Street, Suite 6
Williamsville, New York 14221

Dear Mr. Moynihan:

Please refer to your Pre-Investigational New Drug Application (PIND) file for X-22 low nicotine cigarette.

We also refer to the meeting between representatives of your firm and the FDA on January 21, 2009. The purpose of the meeting was to provide guidance on the development of a low nicotine cigarette for use as a smoking cessation therapy.

A copy of the official minutes of the meeting is attached for your information. Please notify us of any significant differences in understanding regarding the meeting outcomes.

If you have any questions, please call Mary Lewis, Project Manager at (301)796-0941.

Sincerely,

{See appended electronic signature page}

Andrea Leonard-Segal, M.D.
Director
Division of Nonprescription Clinical Evaluation
Office of Nonprescription Products
Center for Drug Evaluation and Research

Enclosure - Meeting Minutes

MEMORANDUM OF MEETING MINUTES

MEETING DATE: January 21, 2009

TIME: 3:00-4:00pm

LOCATION: FDA/White Oak
10903 New Hampshire Avenue
Room 1415
Silver Spring, MD 20993

APPLICATION: PIND 103,589

DRUG NAME: X-22 Low Nicotine Cigarette

TYPE OF MEETING: Type B

MEETING CHAIR: Andrea Leonard-Segal, M.D.
Director, Division of Nonprescription Clinical Evaluation

MEETING RECORDER: Mary M. Lewis
Regulatory Project Manager

FDA ATTENDEES:

Office of Nonprescription Products
Charles J. Ganley, M.D., Director
Leah A. Christl, Ph.D., Associate Director for Regulatory Affairs

Division of Nonprescription Clinical Evaluation
Andrea Leonard-Segal, M.D., Director
Joel Schiffenbauer, M.D., Deputy Director
Priscilla Callahan-Lyon, M.D., Medical Officer
Capt. Laura E. Shay, R.N., M.S., C-ANP, Social Science Analyst
Oluwamurewa Oguntimein, M.H.S., C.H.E.S., Social Science Analyst
Wafa Harrouk, Ph.D., Pharmacology-Toxicology Reviewer
Cindy Li, Ph.D., Pharmacology-Toxicology Reviewer
Mary M. Lewis, R.N., Regulatory Project Manager
Larry Bauer, R.N., M.A., Regulatory Project Manager
Capt. Michelle Poindexter, R.N., B.S.N., M.S.-WHNP-c, Regulatory Project Manager
Melissa Hancock Furness, Chief, Project Management Staff

Division of Anesthesia, Analgesia, and Rheumatology Products
Celia Winchell, M.D., Team Leader
Jane Filie, M.D., Medical Officer

Office of Clinical Pharmacology
David J. Lee, Ph.D., Pharmacologist, Clinical Pharmacology Reviewer

Botanical Review Team, Office of Drug Evaluation I (ODE-1)
Shaw T. Chen, M.D., Botanical Review Team Leader, Associate Director of ODE-I
Jinhui Dou, Ph.D., Pharmacologist

Office of New Drug Quality Assessment
Shulin, Ding, Ph.D., Chemist, Pharmaceutical Assessment Lead

22nd CENTURY LIMITED, LLC:

Joseph Pandolfino, M.B.A., President
Michael R. Moynihan, Ph.D., Vice President, Research & Development
(b) (4) Consultant
(b) (4) Regulatory Consultant

1.0 BACKGROUND:

22nd Century Limited, LLC (22nd Century) submitted a meeting request to the FDA on September 19, 2008 to obtain guidance on the development of a low nicotine cigarette, X-22, as an aid to smoking cessation.

2.0 MEETING OBJECTIVES:

The specific meeting objectives were the following:
(a) Discussion of effects of smoking very low nicotine cigarettes vs. reduced nicotine cigarettes
(b) Discussion of the study design for a Phase II clinical trial of X-22 very low nicotine cigarettes for smoking cessation
(c) Discussion of the proposed contents of the IND including CMC and preclinical information and whether the planned contents of the IND will support the proposed clinical trial
(d) Discussion of rationale for developing X-22 as a prescription product

3.0 DISCUSSION:

Preliminary responses to the questions enclosed in the December 17, 2008 Meeting Package were sent to 22nd Century via email on January 16, 2009.

Following introductions, the meeting agenda consisted of further discussion based on the preliminary responses from the FDA. The questions from 22nd Century appear below followed by the preliminary FDA responses in italics. For questions where no additional discussion is indicated, neither 22nd Century nor FDA raised any additional issues pertaining to the questions.

3.1 QUESTIONS AND FDA PRELIMINARY RESPONSES:

FDA Preliminary Statement:

This product has the "same physical and chemical properties as conventional cigarettes, except for the reduction in nicotine and related compounds." It poses the same risks to users including "cancer, cardiovascular disease, respiratory disease, and reproductive effects." Consumers may recognize this as a "FDA approved" cigarette and could be under the false assumption that it is a "safer cigarette" because it is FDA approved. There are many nicotine replacement therapies available that do not have the risks associated with them that this product poses.

FDA has not yet formulated a definitive opinion regarding the approval of a cigarette as a pharmacologically active product intended for smoking cessation. Risk/benefit issues raise conceptual questions about whether a cigarette could be determined to be safe and effective.

Question 1:

Does FDA agree that this information will be sufficient to evaluate the proposed use of the materials in a Phase II trial with confirmed smokers?

FDA Preliminary Response:

For the proposed Phase 2 trial, the standard supportive preclinical studies required by ICH M3 guidelines could be waived due to the botanical nature of this product and the extensive human use of nicotine in cigarette smoke. However, you should provide the Agency with a comparison of the composition of the smoke generated from the X-22 tobacco with that from commonly used cigarettes smoke. Any novel compound(s) or increased exposure to a compound(s) in the X-22 tobacco or its smoke will require the identification of such compound(s) and may require further toxicological assessment including general toxicology, genetic toxicology, reproductive toxicology and carcinogenicity studies as per the ICH M3 guidelines.

The chemistry information will be sufficient for the proposed Phase II trial. However, we have the following additional comments:

- *You need to develop proper identity tests for botanical raw material (tobacco leaves), botanical drug substance (processed tobacco leaves), and drug product (cigarettes), and implement adequate controls over identity before Phase 3.*

- *We recommend that you conduct a comprehensive chemical characterization on the drug substance and drug product before Phase 3. The information will be valuable in specification setting for Phase 3 supplies.*

- *Provide a comparison of the chemical composition of the smoke and the tar between the proposed product and the non-genetically engineered, commonly used cigarettes.*

- *Propose controls (e.g. measurement of physical attributes or pressure drop) to ascertain the performance of the proposed product, e.g., dose delivery.*

- *Add tests on moisture and microbial limit (USP <61>) to drug product specification before Phase 3. We may request other tests for Phase 3 clinical supplies when the information from a comprehensive chemical characterization study becomes available.*

Additional Discussion:

FDA confirmed again that the CMC information described in the meeting package was adequate to open the IND with the proposed Phase 2 study.

22nd Century asked if they could use a reference cigarette (which is not commercially available) for chemical composition comparison between commonly used commercial cigarettes and the proposed product. FDA indicated the sponsor can provide the information, rationale, and justification in the IND for our review.

22nd Century sought clarification for the identity tests on tobacco leaves, processed tobacco leaves and cigarettes. FDA explained that the sponsor should have appropriate tests in place to confirm identity upon receipt of botanical raw material and drug substance, and upon batch release of the proposed drug product. These tests could be physical and chemical such as chemical markers. The sponsor stated they are not aware of a unique marker for X-22 tobacco. FDA can provide guidance in selecting proper methods and markers for identity tests after the sponsor completes a comprehensive characterization on chemical composition of the drug substance and the drug product.

Question 2:

Does FDA have specific concerns about the length of the period of smoking the very low nicotine cigarette?

FDA Preliminary Response:

There are no particular safety concerns with a six-week course of X-22 as compared to conventional cigarettes for the purposes of this study. It is not clear whether this regimen will result in patients successfully quitting smoking. Prolonged or repeated courses of treatment or long-term use of X-22 (as alternative to conventional cigarettes) would raise a concern.

Question 3:

Does FDA consider an active control (cigarette identical in appearance with similar tar yield to X-22 and a typical ratio of nicotine yield to tar yield) to be the appropriate comparator?

FDA Preliminary Response:

If X-22 is to be marketed as a smoking cessation aid, then this is an appropriate comparator for the initial Phase II study.

It could be useful to ask subjects to "guess" theorize their Treatment Group based on their symptoms. This information may be helpful in terms of assessing subject behavior and treatment success.

Additional Discussion:

22nd Century requested additional explanation as to why subjects should be asked to guess whether they were in the treatment group or control group. FDA explained that subjects using the low nicotine cigarettes may experience nicotine withdrawal symptoms and thereby infer what treatment they are on, unblinding the study. If study participants could easily guess which group they belong to, this could influence their behavior and reporting of symptoms for the duration of the study. 22nd Century requested when they would be expected to gather this type of information. FDA replied they should propose this in their study and we would review and provide feedback.

Question 4:

Does FDA consider 4-week continuous abstinence from the last day of the treatment period to be the appropriate primary endpoint for a Phase II smoking cessation study? What additional follow up, if any, does FDA consider necessary to demonstrate efficacy?

FDA Preliminary Response:

The primary endpoint that you propose is adequate. We do not have any comments regarding any additional follow-up for this Phase 2 study.

A study that demonstrates 4 weeks of continuous abstinence is a good start toward the demonstration of efficacy for this product. Four week quit rates, however, are not an accurate prediction of long term abstinence. Demonstration of a longer period of abstinence (even as long as 6 to 12 months) would be better evidence of product efficacy. This could be particularly important for X-22 since use of this product is not without risk.

Two adequate and well- controlled Phase III studies will be needed for demonstration of efficacy.

Question 5:

Does FDA agree that it may be appropriate for X-22 to be evaluated as a prescription product? What effect, if any, would this have on submission of the proposed IND?

FDA Preliminary Response:

The requirements for the initial IND are the same whether it is an OTC or prescription product. Additional studies or information needed for a NDA application can be discussed during an End of Phase II meeting. See 21 CFR 312.

If you believe that the appropriate use and distribution of your product would be more attainable if approved for prescription use only, we agree that your products should be evaluated as such. In this case discussions over the development of this product and the filing of the NDA should be directed to the Division of Analgesia, Anesthesia and Rheumatology Products.

Additional Discussion:

22nd Century wanted to understand if an NDA for a prescription X-22 cigarette would differ from an NDA for an OTC X-22 cigarette. FDA stated that any future NDA for the X-22 cigarette will look different depending upon whether it is submitted as an OTC or prescription product. The chosen designation will have implications for labeling and the potential need for consumer behavior studies. FDA also explained that the Division of Nonprescription Clinical Evaluation (DNCE) as well as the Division of Anesthesia, Analgesia, and Rheumatology Products (DAARP) have been, and will continue to be, involved in the review process for an OTC X-22 cigarette. If the company decides that they would like to pursue a prescription NDA, DNCE will no longer be involved.

FDA commented that this PIND presents the novel concept of using a cigarette in the treatment arena of nicotine addiction and smoking cessation. This concept and approach to smoking cessation would most likely need to be reviewed by an Advisory Committee (AC). The FDA said that such a committee could potentially meet even before the NDA stage. There was a discussion as to whether such an AC meeting would be closed or open and FDA stated that this would be decided at the time the content of the meeting was more clearly defined. If such a committee is formed, 22nd Century will likely be asked to provide information for the committee to review. FDA also stated regardless as to whether an AC meeting is held during the IND stage, that an AC meeting would likely be held after the NDA is submitted during the course of the 10-month review cycle.

22nd Century stated that their cigarette contains 95% less nicotine than regular U.S. tobacco and that research shows that people eventually smoke less when smoking cigarettes with very low nicotine. They commented that the amount of nicotine in their cigarette is low but measurable. The FDA stated that 22nd Century would need to demonstrate that the nicotine exposure from their product is not more bioavailable than nicotine from other approved nicotine replacement products, or the sponsor would need to provide adequate safety data to support the difference. It is possible that the bioavailability profile of this product, which would be different from other smoking cessation therapies (smoked cigarette vs. nicotine replacement therapy such as oral or patch). This difference will have to be considered when evaluating the safety of this product. 22nd Century stated that they had not considered what the highest dose of nicotine from their cigarette might be and that they would further consider the added comments supplied today.

FDA also stated that 22nd Century would need to consider what the dosing regimen for their drug product, the X-22 cigarette, would be. The sponsor acknowledged this.

22nd Century asked for clarification regarding when clinical pharmacology wanted the studies in Attachment A submitted, and FDA stated that the exposure information should ideally be obtained early in the development program so that this information can be appropriately taken into account before larger phase III clinical trial(s) are undertaken. FDA stated that the sponsor has an option to conduct clinical pharmacology studies as either stand-alone studies or included as part of any clinical study proposed, in order to obtain the needed information stated in Attachment A.

FDA stated that there would be a need for consumer instructions as they relate to dosing, a tapering plan, and general usage. These instructions may affect how the sponsor performs future studies including consumer behavior studies.

Botanical Issues - The sponsor asked about meeting the requirements of product quality and therapeutic consistency as a botanical product. FDA advised them to consult the FDA's Botanical Guidance, the available reviews of NDA 21-902, the only botanical NDA that has thus far been approved, and an article published by the Agency's Botanical Review Team (in Nature Biotechnology, October 2008). Further discussion on this issue with ONDQA and the Botanical Team may be necessary.

4.0 UNRESOLVED ISSUES OR ISSUES REQUIRING FURTHER DISCUSSION:

- 22nd Century will consider whether they will pursue prescription or OTC status for X-22.
- FDA is unclear as to whether a cigarette could be approved as a drug for smoking cessation because of the complicated risk/benefit issues such a concept raises.

5.0 SUMMARY OF AGREEMENTS AND ACTION ITEMS:

22nd Century will justify their choice of a reference cigarette when they submit their IND.

After 22nd Century comprehensively characterizes the chemical composition of the drug substance and drug product, FDA will be able to provide guidance in selecting proper methods and markers for identity tests.

In their study protocol, 22nd Century will propose when they will gather information on whether study subjects correctly guessed if they were in the control or treatment group and FDA will provide comments.

22nd Century understands that the content of their NDA will differ depending upon whether they submit and NDA for a prescription product or for an OTC product.

The sponsor understands that they need to provide the Clinical Pharmacology data listed in Attachment A and that this information should be provided early in the clinical development

program. The sponsor understands that they will need to provide data to justify the safety of the nicotine exposure their product delivers if it exceeds that of approved NRT products

The sponsor understands that they need to provide a dosing regimen for their drug product.

The sponsor will consult the Botanical Guidance, the one approved botanical NDA, and the October 2008 article published in Nature Biotechnology to learn more about meeting the quality and therapeutic consistency requirements of a botanical product.

The sponsor will write the consumer materials that they would need to pursue consumer studies and will provide them to FDA for comment.

It is likely that more than one Advisory Committee meeting may be needed to consider this drug product. One AC meeting may occur during the IND stage and another during the NDA review period.

6.0 ATTACHMENTS

Attachment A
Additional Comments: During the meeting with the sponsor on January 21, 2009; a handout was provided to 22nd Century from the FDA Clinical Pharmacology Reviewer, David J. Lee, Ph.D.

Attachment A

Pre IND 103,589
X-22 Low Nicotine Cigarette, XX22 Century LTD.
For meeting January 21, 2009

Clinical Pharmacology Additional Comments:

Based on our current understanding of your product, you need to determine the following X-22 pharmacokinetic characteristics:

1. Relative bioavailability (X-22 vs. a U.S. approved nicotine product as a comparator, e.g., a gum, lozenge, or inhaler)
2. Multiple dose pharmacokinetics:
    a. After maximum likely use (nicotine PK information from the most intensive dosage regimen to measure the highest plasma levels that can be achieved)
    b. After a standard regimen (for example, every hour for up to 12 hours)

Linked Applications	Sponsor Name	Drug Name / Subject
IND 103589	22nd Century Limited, LLC	X-22 Smoking Cessation Product

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ANDREA LEONARD SEGAL
02/19/2009

APPENDIX 2

RESPONSE TO FDA COMMENTS FROM MINUTES OF JANUARY 21, 2009 PRE-IND MEETING

Response to FDA Pre-IND Comments

Reference is made to 22nd Century's Pre-IND 103,589 submission on December 27, 2008. Reference is also made to our January 21, 2009, pre-IND meeting with members of the Office of Nonprescription Products, the Division of Anesthesia, Analgesia and Rheumatology Products, the Office of New Drug Quality Assessment, the Division of Nonprescription Clinical Evaluation, the Office of Clinical Pharmacology, and the Botanical Review Team of the Office of Drug Evaluation 1 to discuss the Initial IND submission package for X-22 Smoking Cessation Product. Furthermore, reference is also made to FDA's official minutes of the meeting dated and received on February 19, 2009.

We have considered all FDA comments/suggestions and incorporated them into the IND application documentation and/or into our future development plans, as appropriate.

This document provides our responses to the FDA's Summary of Agreements and Action Items (Item 5) from the FDA's official minutes of the meeting. The FDA comments are repeated in **Bold,** followed by 22nd Century's responses. Within the 22nd Century responses, the respective information and/or reference to documents in the IND submission is given.

FDA Summary Comments from Pre-IND Meeting:

For the proposed Phase 2 trial, the standard supportive preclinical studies required by ICH M3 guidelines could be waived due to the botanical nature of this product and the extensive human use of nicotine in cigarette smoke. However, you should provide the Agency with a comparison of the composition of the smoke generated from the X-22 tobacco with that from commonly used cigarettes smoke. Any novel compound(s) or increased exposure to a compound(s) in the X-22 tobacco or its smoke will require the identification of such compound(s) and may require further toxicological assessment including general toxicology, genetic toxicology, reproductive toxicology and carcinogenicity studies as per the ICH M3 guidelines.

22nd Century Response:

Information on the composition of the smoke generated from Magic® very low nicotine (VLN) cigarettes (manufactured from the same VLN tobacco variety (Vector 21-41) used in X-22) is summarized in Item 8, Section 3.1.2 of the IND, and the report is included in Item 8. The comparison was made against top-selling commercial brands (Marlboro® Gold Box King, Newport® Menthol and Camel® Blue Box King).

Information is also included in the IND on the composition of the smoke generated from the same VLN tobacco variety (Vector 21-41), used in X-22 Smoking Cessation Product, but formulated into a different VLN cigarette (Quest 3®). This information is summarized in Item 8, Section 3.1.3 of the IND, and the report is included in Item 8. The comparison was made against two top-selling commercial brands (Marlboro Light and Camel 100), and the KY2R4F reference cigarette (a tobacco industry analytical standard cigarette).

The chemistry information will be sufficient for the proposed Phase II trial. However, we have the following additional comments:

• You need to develop proper identity tests for botanical raw material (tobacco leaves), botanical drug substance (processed tobacco leaves), and drug product (cigarettes), and implement adequate controls over identity before Phase 3.

22nd Century Response:

22nd Century will develop appropriate identify tests for the botanical raw material, botanical drug substance and drug product and provide these to the Agency for review and comment prior to commencement of Phase 3 clinical trials.

• We recommend that you conduct a comprehensive chemical characterization on the drug substance and drug product before Phase 3. The information will be valuable in specification setting for Phase 3 supplies.

22nd Century Response:

A comprehensive chemical characterization of the botanical drug substance and the X-22 Smoking Cessation Product will be conducted and provided to the Agency for review and comment prior to commencement of Phase 3 clinical trials.

• Provide a comparison of the chemical composition of the smoke and the tar between the proposed product and the non-genetically engineered, commonly used cigarettes.

22nd Century Response:

As stated above, information on the chemical composition of the smoke and tar generated from Vector 21-41 tobacco used in the X-22 cigarette is summarized in Item 8, Section 3.1.2 of the IND, and the report is included in Item 8. Furthermore, presented in Table 2-1 of Item 8 of the IND are data on the physical and chemical characteristics of the X-22 clinical trial cigarettes, including machine smoke yields, compared to (i) other very low nicotine (VLN) cigarettes (Magic Menthol and Quest 3 Menthol), both of which are manufactured from the same VLN tobacco variety (Vector 21-41) used in X-22 cigarettes, (ii) Newport® Menthol and Marlboro® Gold, two of the top selling brand styles in the U.S., (iii) the KY2R4F Reference Cigarette, a tobacco industry standard (typical nicotine) cigarette, and (iv) the Active Control cigarette for Study X-22-201.

• Propose controls (e.g. measurement of physical attributes or pressure drop) to ascertain the performance of the proposed product, e.g., dose delivery.

22nd Century Response:

22nd Century will consider proposed controls to ascertain the performance of X-22 Smoking Cessation Product and provide these to the Agency for review and comment prior to commencement of Phase 3 clinical trials.

• Add tests on moisture and microbial limit (USP <61>) to drug product specification before Phase 3. We may request other tests for Phase 3 clinical supplies when the information from a comprehensive chemical characterization study becomes available.

22nd Century Response:

22nd Century will develop proposed moisture and microbial limits for X-22 Smoking Cessation Product and provide these to the Agency for review and comment prior to commencement of Phase 3 clinical trials.

FDA Summary of Agreements and Action Items from the Pre-IND Meeting:

#1 22nd Century will justify their choice of a reference cigarette when they submit their IND.

22nd Century Response:

22nd Century had originally proposed a comparison of the X-22 cigarette to a reference cigarette in mainstream smoke chemistry studies, instead of comparing it to leading commercial brands, however, we have been able to compare the X-22 cigarette against both a reference cigarette and top-selling commercial brands in such studies. Data from these studies is contained in the IND in Item 8, Sections 3.1.2 and 3.1.3.

The rationale for inclusion of a reference cigarette is that commercial cigarettes vary in their design, and individual brand styles may vary over time due to variation in weather and other crop conditions, as well as design changes. The reference cigarette program was initiated in the late 1960s at the University of Kentucky to provide a standard for research purposes. In 1983 the 1R4F cigarette, with FTC machine smoking yields similar to "light" cigarettes (the most popular style in the U.S.) was developed through a joint effort of the National Cancer Institute (NIH), Agriculture Research Service-USDA and the Tobacco and Health Research Institute of the University of Kentucky. 2R4F and 3R4F are subsequent lots.

#2 After 22nd Century comprehensively characterizes the chemical composition of the drug substance and drug product, FDA will be able to provide guidance in selecting proper methods and markers for identity tests.

22nd Century Response:

22nd Century appreciates the FDA's offer to provide guidance in selecting the proper methods and markers for identity tests.

#3 In their study protocol, 22nd Century will propose when they will gather information on whether study subjects correctly guessed if they were in the control or treatment group and FDA will provide comments.

22nd Century Response:

In Study X-22-201, subjects will guess which treatment group they were assigned to during Visit 7 (after they have completed the 4-week abstinence period).

#4 22nd Century understands that the content of their NDA will differ depending upon whether they submit an NDA for a prescription product or for an OTC product.

22nd Century Response:

22nd Century appreciates that regulatory requirements will vary depending on whether the NDA is submitted for a prescription product or for an OTC product. 22nd Century has decided to develop its investigational product, X-22 Smoking Cessation Product, for the indication of smoking cessation as a prescription product.

#5 The sponsor understands that they need to provide the Clinical Pharmacology data listed in Attachment A and that this information should be provided early in the clinical development program. The sponsor understands that they will need to provide data to justify the safety of the nicotine exposure their product delivers if it exceeds that of approved NRT products.

Attachment A

Clinical Pharmacology Additional Comments:

Based on our current understanding of your product, you need to determine the following X-22 pharmacokinetic characteristics:

1. **Relative bioavailability (X-22 vs. a U.S. approved nicotine product as a comparator, e.g., a gum, lozenge, or inhaler)**

2. **Multiple dose pharmacokinetics:**

   a. After maximum likely use (nicotine PK information from the most intensive dosage regimen to measure the highest plasma levels that can be achieved)

   b. After a standard regimen. (for example, every hour for up to 12 hours)

22nd Century Response:

22nd Century understands the FDA's comment about obtaining X-22 Smoking Cessation Product nicotine exposure information early in clinical development program and plans to do FDA's recommended study before phase III. It is important to note, as shown in Table 2-1 in Item 8 of the IND, that each X-22 cigarette contains approximately 0.3 mg of nicotine (before combustion) and each X-22 cigarette has a machine smoke yield of ≤0.05 mg nicotine. This represents a reduction in nicotine of more than 95% compared to typical commercial cigarettes, including compared to low-yield cigarettes such as those previously labeled "lights" and "ultra lights."

#6 The sponsor understands that they need to provide a dosing regimen for their drug product.

22nd Century Response:

22nd Century understands that we will need to provide a dosing regimen for X-22 Smoking Cessation Product. In Protocol X-22-201, subjects with a smoking history of one-half pack to two packs per day will be allowed to consume study cigarettes as desired for six weeks up to a maximum of approximately 20% more than their pre-study smoking history consumption. Other dosing regimens may be evaluated during the development of X-22 Smoking Cessation Product.

#7 The sponsor will consult the Botanical Guidance, the one approved botanical NDA, and the October 2008 article published in Nature Biotechnology to learn more about meeting the quality and therapeutic consistency requirements of a botanical product.

22nd Century Response:

We have reviewed the Botanical Guidance, the Veregen NDA #021902 and the October 2008 article in *Nature Biotechnology* to better understand the quality and therapeutic consistency requirements of a botanical product. The CMC section of this IND, Item 7, has information relating to these factors for our X-22 Smoking Cessation Product.

#8 The sponsor will write the consumer materials that they would need to pursue consumer studies and will provide them to FDA for comment.

22nd Century Response:

22nd Century will consider this comment during the development of X-22 Smoking Cessation Product and will consult with the FDA as appropriate to develop consumer information materials.

#9 It is likely that more than one Advisory Committee meeting may be needed to consider this drug product. One AC meeting may occur during the IND stage and another during the NDA review period.

22nd Century Response:

22nd Century understands that using a cigarette-based product as a smoking cessation aid is a novel approach to a significant health issue and that one or more Advisory Committees might be needed in order for all affected parties to understand and resolve the complex issues this approach might raise. We look forward to working with FDA during the development process to demonstrate how X-22 Smoking Cessation Product, as a prescription product, can function as an effective therapy to extinguish the smoking habit and not just function as an alternative cigarette product.

INVESTIGATOR'S BROCHURE

X-22 SMOKING CESSATION PRODUCT

Edition: 1.0, 20 June 2011

22nd Century Limited, LLC
8201 Main Street, Suite 6
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Previous Editions:
None

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X-22 SMOKING CESSATION PRODUCT

INVESTIGATOR'S BROCHURE APPROVAL

This Investigator's Brochure has been approved by 22nd Century Limited, LLC. The following signature documents this approval.

(b) (6)

Date: 6/20/11

Michael R. Moynihan, PhD
Vice President, Research & Development

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3. GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

° C	degrees Celsius
° F	degrees Fahrenheit
AD	Alzheimer's Disease
ADHD	Attention Deficit Hyperactivity Disorder
AE	adverse event
APHIS	Animal and Plant Health Inspection Service
bpm	beats per minute
CI	confidence interval
CO	carbon monoxide
CRF	case report form(s)
CRO	Contract Research Organization
CSC	cigarette smoke condensate
DAO	4-aminobutanol by diamine oxidase
DNA	deoxyribonucleic acid
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
EEG	Electroencephalogram
EPA	Environmental Protection Agency
FDA	(U.S.) Food and Drug Administration
FTC	Federal Trade Commission
FTND	Fagerström Test of Nicotine Dependence
g	gram(s)
GCP	Good Clinical Practice (Guidelines)
GWA	genome wide association
HDPE	high-density polyethylene
HHS	Health and Human Services
HR	heart rate
HSD	hemodynamic shearing device
IARC	International Agency for Research on Cancer
IB	investigator brochure
ICH	International Conference on Harmonisation
IND	Investigational New Drug (Application)
IRB	institutional review board

ITT	intent-to-treat (population)
IV	intravenous
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
MOR	mu opiod receptor
ng	nanogram(s)
nAChR	nicotinic acetylcholine receptors
NAB	N-Nitrosoanabasine
NAD	nicotinamide adenine dinucleotide
NAMN	nicotinic acid mononucleotide
NAT	N-Nitrosoanatabine
NCSU	North Carolina State University
NDA	new drug application
NIDA	National Institute on Drug Abuse
NRT	nicotine replacement therapy
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N-9-nitrosonornicotine
PD	Parkinson's Disease
PE	physical examination
PET	positron emission tomography
PP	per protocol (population)
ppm	parts per million
PREP	potential reduced exposure products
QD	once daily
QPTase	quinolinic acid phosphoribosyltransferase
RNA	ribonucleic acid
RNC	reduced nicotine cigarette
RTI	Research Triangle Institute International
SAE	serious adverse event
SBP	systolic blood pressure
SEM	standard error of the mean
SNP	single nucleotide polymorphism
TID	three times daily
TSNA	tobacco-specific nitrosamine

UB	usual brand
USDA	United States Department of Agriculture
VLN	very low nicotine (cigarette)
WHO	World Health Organization
µg	microgram(s)

4. SUMMARY

The X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product in the form of a cigarette that is being developed for the indication of smoking cessation. X-22 cigarettes, like conventional cigarettes, consist of filler, filter, and wrapper components. X-22 cigarettes contain menthol, (b) (4) The menthol is included in part to compensate for the reduced sensory impact of the cigarettes in the mouth and throat due to the very low nicotine content.

The filler of X-22 cigarettes contains a proprietary very low nicotine (VLN) tobacco variety, Vector 21-41, which has very low levels of nicotine due to genetic modification of the nicotine biosynthesis pathway in the plant.[1] X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of <1 mg/g and a machine smoking yield ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes.

All processing of tobacco is carried out by processors of conventional tobacco. All manufacturing of cigarette components and cigarettes is carried out by manufacturers of conventional cigarette components and cigarettes. The composition of smoke from X-22 cigarettes is very similar to the composition of smoke from conventional cigarettes.[2] The product is intended to reduce cravings for cigarettes and increase the chances of successfully quitting smoking.

5. INTRODUCTION

22nd Century intends to develop its investigational product, X-22 Smoking Cessation Product, for the indication of smoking cessation. X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product in the form of a cigarette. Like conventional cigarettes, X-22 cigarettes consist of filler, filter, and wrapper components. X-22 cigarettes contain menthol, (b) (4) The filler of X-22 cigarettes contains a proprietary very low nicotine (VLN) tobacco variety, Vector 21-41, which has very low levels of nicotine due to genetic modification of the nicotine biosynthesis pathway in the plant.[1] X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of <1 mg/g and a nicotine yield of ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes.

5.1. Medical Need

It has been well established that cigarette smoking is toxic and poses health risks to subjects, namely cardiovascular disease, multiple cancers, pulmonary disease, adverse reproductive outcomes, and exacerbation of other chronic health conditions.[3] Cigarette smoking is the leading cause of preventable morbidity and mortality in the United States, causing approximately 443,000 premature deaths annually.[4] In 2009, 20.6% of adults in the United States were current cigarette smokers.[4] It is the goal of the US Department of Health and Human Services, as outlined in the program Healthy People.gov, to reduce cigarette smoking in the United States to 12% by the year 2020. This goal was previously set for, but not attained in, the year 2010.[5]

Cigarette smoking is a complex behavior that is sustained by the pharmacological properties of nicotine and reinforcing behavioral and sensory cues.[6,7] Smokers report reduced satisfaction when sensory signals are blocked or altered using methods such as local airway anesthesia[8] and addition or elimination of menthol.[9] Furthermore, nicotine delivered intravenously in the absence of the sensory and behavioral aspects of cigarette smoking is rated a less satisfying experience than delivery via inhalation from a cigarette.

Currently available pharmacological approaches for smoking cessation in combination with counseling and education (maximal intervention) are effective in a minority of smokers, with the probability of initial success with all types of approved pharmacotherapy increased 1.5 to 2.5 fold compared to placebo.[10,11] Relapse is common following use of approved therapies, and 75 to 80 percent of smokers who receive treatment are unable to achieve long-term abstinence at 6 or 12 months.[12,13] Therefore, there is an important public health need to provide more effective smoking cessation treatments, reduce relapse, and to provide alternative treatments for smokers who may not succeed with, or do not have access to, treatment that includes approved pharmacotherapies.

5.1. X-22 Smoking Cessation Product

The X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product in the form of a cigarette. X-22 cigarettes, like conventional cigarettes, consists of filler, filter, and wrapper components. X-22 cigarettes contain menthol, (b) (4) The filler in X-22 cigarettes, like that of conventional cigarettes, is comprised primarily of cured tobacco leaf and does not contain non-tobacco plant material (other than a relatively small amount present in some of the additives and flavorings included in the filler). The filler of X-22 cigarettes contains a proprietary VLN tobacco variety, Vector 21-41. All processing of tobacco is carried out by processors of conventional tobacco. All manufacturing of cigarette components and cigarettes is carried out by manufacturers of conventional cigarette components and cigarettes.

X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of less than 1 mg/g and a machine smoking yield of ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes. Except for the use of the VLN tobacco variety, the growing, curing and processing of tobacco for X-22 cigarettes are the same as for conventional cigarettes, and X-22 cigarettes have the same physical and chemical properties as conventional cigarettes, except for the reduction in nicotine and related compounds. The composition of smoke from X-22 cigarettes is very similar to the composition of smoke from conventional cigarettes.[2]

5.2. Scientific Rationale for the Development of a Cigarette Manufactured with Very Low Nicotine Tobacco

Cigarettes containing very low levels of nicotine consistently reduce craving for cigarettes and withdrawal in smokers.[14,15,16,17,18,19,20,21,22,23] In contrast to conventional low-yield ("light" or "ultra light") cigarettes that have reduced tar and nicotine yields mainly due to smoke dilution, smoke from cigarettes containing very low levels of nicotine contains greatly reduced ratios of nicotine to other smoke components.[2] The data from studies of the behavioral and pharmacodynamic properties of cigarettes containing very low levels indicate that a product that delivers the sensory and behavioral cues associated with cigarette use but does not deliver a satisfying amount of nicotine breaks the association of those cues with the contingent delivery of nicotine reinforcement and promotes extinction of smoke cues.

Much of the early research cited in the paragraph above was conducted with denicotinized cigarettes. Denicotinized cigarettes differ from cigarettes such as X-22 cigarettes in that denicotinized cigarettes are manufactured with conventional tobacco from which nicotine has been removed by chemical processes. Several types of denicotinized cigarettes have been produced in the past several decades. In 1989, Philip Morris Inc. (Richmond, VA) tested the market acceptability of a denicotinized cigarette (Next®). Nicotine was removed from the tobacco in Next using high-pressure carbon dioxide. Although the cigarette was not well accepted in the retail market, it proved useful in clinical studies to estimate the effects of smoking with minimal nicotine delivery.

In response to the need among tobacco researchers for a cigarette containing very low levels of nicotine, the National Institute on Drug Abuse (NIDA) contracted with a private firm (Ultratech/Lifetech Corporation, Lafayette Hill, PA) to develop and manufacture denicotinized cigarettes. According to Pickworth *et al*[17], the denicotinized cigarettes had the nicotine removed with a mild alkaline (ammonium solution) wash and had similar characteristics as commercial cigarettes, but contained and delivered very low nicotine. Four types of cigarettes were developed and tested: reduced- and full-tar standard cigarettes and identically appearing reduced- and full-tar denicotinized cigarettes that delivered carbon monoxide (CO) and tar, but very small amounts of nicotine.

Processes involved in removing nicotine from tobacco have been found not to be specific for nicotine and other leaf components are also removed, resulting in changes in taste and other factors.[24] Levels of monoamine oxidase inhibitors (MAOIs) such as β-carboline alkaloids, naphthoquinone, and acetaldehyde are also affected by extracting nicotine from tobacco. Besides nicotine, the following compounds or compound classes have been known to be simultaneously extracted from denicotinized tobacco:

- Isoprenoids, a class of compounds that are acyclic terpenes such as solanesol and neophytadiene.
- Oils and waxes, which cover a number of compound classes and a larger number of specific compounds analogous to essential oils in other plants such as citrus fruit, these compounds in tobacco are largely responsible for the characteristic "tobacco" flavor and aroma. Removal of these compounds, or even a change in the concentration ratios, can destroy the characteristic flavor or aroma of a plant, including tobacco.
- Carboxylic Acids - Tobacco contains many, however, palmitic acid, oleic acid, and a number of C18-fatty acids were specifically identified.
- Hydrocarbons in the range C27H50 to C33H68.
- Other compounds, including cembranoids and stigmasterol.

In contrast to denicotinized tobacco, the tobacco used in X-22 cigarettes is produced from a VLN tobacco variety that when grown to maturity using conventional practices contains approximately 95% less nicotine than conventional tobacco plants. X-22 cigarettes may therefore offer benefits relative to cigarettes manufactured with conventional tobacco that has had nicotine chemically removed.

Vector 21-41 has been used in several other VLN cigarette formulations. Recently it was used to prepare the Magic® VLN cigarette. This cigarette differs from X-22 cigarettes only in the flavoring and wrapper characteristics. Data from a smoke composition study with Magic is presented in Section 6.1.2.

Quest® cigarettes, manufactured using the same VLN tobacco as X-22 cigarettes, were sold by Vector Tobacco Inc. from December 2002 to December 2007, and Quest Menthol® cigarettes were sold by Vector Tobacco Inc. from November 2003 through December 2007. Consumers purchased these Quest and Quest Menthol cigarettes for at least another year. Both Quest and Quest Menthol cigarettes were produced in brand styles containing 3 levels of nicotine in the tobacco filler, resulting in the following nicotine yields per cigarette: Quest 1® (0.60 milligrams [mg]), Quest 2® (0.3 mg) and Quest 3® (≤0.05 mg). In addition to being sold commercially, Quest 3 cigarettes have been used in clinical studies, including trials evaluating their usefulness as an aid to smoking cessation. These trials are described in Section 10.2.

Most recently, NIDA in 2009 included an option to develop and produce research cigarettes with different levels of nicotine (from very low to high), in its request for proposals for a 5-year contract for Preparation and Distribution of Research and Drug Products. 22nd Century agreed, as a subcontractor to Research Triangle Institute International (RTI), in RTI's contract with NIDA for the Research Cigarette Option, to supply 9 million cigarettes having 8 different nicotine contents to NIDA. These research cigarettes, Spectrum™, are expected to be delivered to RTI in July of 2011.

6. PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

X-22 is administered by combustion and inhalation of its smoke. X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of less than 1 mg/g and a machine smoking yield of ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes and the University of Kentucky Reference 2R4F (KY2R4F) cigarette (a tobacco industry analytical reference standard). A comparison of some of the physical and chemical characteristics of X-22 cigarettes, Magic Menthol, Quest 3 Menthol, Newport Menthol, Marlboro Gold, a tobacco industry standard (normal nicotine) cigarette (KY2R4F), and the active control cigarette to be used in X-22 clinical trials is presented in Table 6-1.

Table 6-1 Comparison of X-22, Magic Menthol, Quest 3 Menthol®, Newport® Menthol, Marlboro® Gold, KY2R4F Reference, and Active Control Cigarettes

	X-22	Magic Menthol	Quest 3 Menthol	Newport Menthol	Marlboro Gold	KY2R4F	Active Control
Chemical:							
Filler Composition							
Nicotine (mg/g)	0.5	1.9	1.2	19	18	18	21
Nicotine (mg/cigarette)	0.3	1.2	0.7	14	12	14	16
Smoke yields							
"Tar" (mg/cigarette)	7	4	11	19	10	10	9
Nicotine (mg/cigarette)	≤0.05	≤0.05	≤0.05	1.1	0.7	0.8	0.7
CO (mg/cigarette)	13	5	15	20	10	12	12
Physical:							
Total Weight (mg/cigarette)	970	940	860	900	917	1060	1010
Filler Weight (mg/cigarette)	680	630	580	740	660	780	770
Length (mm)	83	83	83	80	83	84	83
Circumference (mm)	24.6	24.6	25.0	25	24.8	24.8	24.6
Pressure Drop (mm H_2O)	125	81	116	128	118	128	120

6.1. Comparison of X-22 Cigarettes to Conventional Cigarettes

6.1.1. Smoke Chemistry

Tobacco is unique among commercial botanical products in that its primary use (i.e., in the form of cigarettes) is to supply, upon combustion and subsequent inhalation, an aerosolized mixture of gaseous and suspended particulate components to the consumer. As with any botanical, the naturally complex chemical composition of the tobacco leaf is increased dramatically upon combustion. In addition to nicotine, there are approximately 4800 individual smoke constituents that have been identified in currently marketed United States cigarettes. The International Agency for Research on Cancer (IARC) and the Environmental Protection Agency (EPA) have identified over 70 compounds in the

mainstream smoke of cigarettes as known, probable, or possible carcinogens. The Tobacco Products Scientific Advisory Committee of the FDA Center for Tobacco Products recently compiled a list of 107 harmful and potentially harmful components of cigarette smoke. Toxicological and analytical data for the vast majority of these constituents individually do not yet exist. Even for those components for which such data do exist, there is currently no accepted national standard for assessing individual exposure or for defining maximal permissible exposures, nor is there agreement as to either the relative chemical toxicity or biological activity for individuals exposed to these substances, alone or in combination. Currently, there is no requirement to collect data on individual smoke constituents.

Despite the lack of a cohesive and codified regimen for qualitatively and quantitatively assessing the full complement of toxic and carcinogenic substances in cigarette smoke, there is at present a generally accepted list of approximately 45-55 tobacco constituents for which sufficient evidence exists of a prominent health risk to consumers. The genesis of this list is based on the work of Dr. Dietrich Hoffmann. This list, which has been periodically revised and updated since its introduction by Dr. Hoffmann, is used as an unofficial benchmark guide to develop new testing regimens for exposure to cigarette toxins and carcinogens by several major national [i.e., the EPA and the Occupational Safety and Health Administration (OSHA)] and international agencies [i.e., IARC and the World Health Organization (WHO)] devoted to protecting the health of the public. In addition, in North America, Canada has passed legislation that requires the annual testing and reporting of over 40 cigarette smoke constituents for all cigarette brands sold. Most, if not all, of these constituents are contained within the 'Hoffmann' list of cigarette toxins and carcinogens or one of its variants.[25,26]

6.1.2. Comparison of Smoke from Cigarettes Made with VLN Tobacco with Smoke from Commercial Cigarettes

6.1.2.1. Filtrona Technology Centre 2011 Smoke Chemistry Analysis

In order to provide a systematic characterization of the principal combustion components of cigarettes made from Vector 21-41 tobacco, Filtrona Technology Centre was contracted to perform an analysis of mainstream smoke constituents[27] and assess the levels of individual toxins and carcinogens derived primarily from the 'Hoffmann' list. This analysis compared smoke from the following cigarettes:

- Magic Menthol, a cigarette manufactured by 22nd Century that has a formulation very similar to X-22 cigarettes, including the same VLN tobacco variety (Vector 21-41) used in X-22 cigarettes,
- Magic, a non-menthol version of Magic Menthol,
- Newport Menthol King, a top-selling U.S. commercial cigarette style,
- Marlboro Gold King, the top-selling U.S. commercial cigarette style, and

- Camel® Blue, a top-selling U.S. commercial cigarette style.

Smoke was analyzed for a spectrum of well-known toxins and carcinogens typically found in combusted cigarettes.

The results of this analysis are provided in Table 6-2. These results confirm that the smoke from the VLN (Magic Menthol) cigarette made with the Vector 21-41 tobacco used in X-22 cigarettes is chemically similar to smoke from conventional cigarettes.

Table 6-2 Mainstream Smoke Chemistry Data[1] Conducted by Filtrona Technology Centre on the Magic Formulation

Analyte Tested	Magic Menthol®	Magic®	Newport Menthol®	Marlboro Gold®	Camel Blue®
Tar, Nicotine, CO (mg/cigarette)					
Tar	3.5 (0.2)	3.7 (0.2)	18.7 (0.7)	10.1 (0.3)	10.8 (0.9)
Nicotine	0.03 (0.00)	0.03 (0.00)	1.07 (0.02)	0.72 (0.02)	0.80 (0.05)
Carbon Monoxide	5.4 (0.2)	6.3 (0.4)	19.7 (0.7)	10.5 (0.2)	12.5 (0.8)
Ammonia (µg/cigarette)	24.8 (2.0)	23.1 (2.1)	14.0 (0.2)	9.9 (2.0)	9.5 (0.3)
Aromatic Amines I (ng/cigarette)					
Aminobiphenyl, 3-	1.50 (0.13)	1.55 (0.09)	0.83 (0.09)	0.99 (0.07)	0.91 (0.01)
Aminobiphenyl, 4-	1.02 (0.07)	0.89 (0.09)	0.61 (0.10)	0.69 (0.11)	0.55 (0.08)
Aminonaphthalene, 1-	7.6 (0.1)	8.3 (0.2)	10.5 (0.4)	11.2 (0.4)	9.9 (0.4)
Aminonaphthalene, 2-	4.16 (0.08)	4.38 (0.11)	2.32 (0.12)	2.97 (0.40)	2.70 (0.24)
Carbonyls/Aldehydes (µg/cigarette)					
Acetaldehyde	283 (13)	353 (41)	768 (29)	474 (11)	563 (13)
Acetone	112 (8)	138 (19)	265 (12)	167 (3)	202 (5)
Acrolein	12.0 (0.9)	14.8 (2.4)	75.2 (1.2)	43.6 (1.9)	48.9 (4.3)
Butryaldehyde	10.4 (1.1)	13.2 (1.2)	39.9 (4.3)	26.2 (1.4)	30.3 (0.3)
Crotonaldehyde	4.6 (0.8)	7.5 (0.8)	16.9 (1.2)	8.9 (0.5)	16.4 (1.1)
Formaldehyde	5.3 (1.5)	5.7 (0.6)	33.0 (1.0)	17.7 (1.5)	19.3 (2.5)
Methylethylketone	23.4 (2.2)	28.4 (2.4)	55.5 (3.2)	35.7 (1.1)	45.1 (2.7)
Propionaldehyde	20.0 (1.3)	25.3 (3.0)	53.7 (1.9)	34.7 (0.7)	42.4 (0.9)
Hydrogen Cyanide (µg/cigt)	44 (5)	60 (4)	291 (22)	137 (2)	158 (12)
Menthol (mg/cigarette)	0.34 (0.01)	not detected	0.58 (0.2)	not detected	not detected
Metals (ng/cigarette)					
Arsenic	<1.5	<1	4.3 (0.3)	3.1 (0.1)	2.8 (0.3)
Beryllium	<2	<2	<2	<2	<2
Cadmium	13.8 (1.3)	16.8 (0.4)	63.3 (1.4)	36.7 (1.4)	40.8 (1.4)

Analyte Tested	**Magic Menthol®**	**Magic®**	**Newport Menthol®**	**Marlboro Gold®**	**Camel Blue®**
Chromium	<10	<10	<10	<10	<10
Cobalt	<2	<2	<2	<2	<2
Lead	<5	5.3 (0.4)	21.2 (0.1)	14.3 (0.3)	14.3 (0.7)
Nickel	<10	<10	<10	<10	<10
Selenium	<2	<2	<2	<2	<2
Nitric Oxide (µg/cigarette)	153 (3)	200 (7)	163 (12)	155 (6)	156 (5)
Phenols (µg/cigarette)					
Catechol	13.2 (0.3)	14.1 (1.0)	59.0 (0.8)	40.8 (0.6)	41.3 (2.2)
Cresol, m- & p-	3.9 (0.2)	4.5 (0.3)	9.2 (0.4)	7.0 (0.2)	6.1 (0.4)
Cresol, o-	1.5 (0.1)	1.7 (0.1)	3.6 (0.2)	2.8 (0.2)	2.3 (0.2)
Hydroquinone	16 (1)	18 (2)	64 (2)	38 (2)	38 (3)
Phenol	5.1 (0.3)	6.3 (0.3)	13.4 (0.7)	10.6 (0.2)	8.8 (0.5)
Resorcinol	<0.5	<0.5	5.7 (0.3)	3.2 (0.1)	3.5 (0.2)
Polynuclear Aromatic Hydrocarbons (ng/cigarette)					
Benzo[a]pyrene	0.9 (0.1)	2.4 (0.1)	9.3 (0.1)	9.7 (0.2)	6.7 (0.7)
Semi-Volatiles (µg/cigarette)					
Pyridine	3.5 (0.3)	6.0 (0.4)	18.0 (1.2)	12.6 (1.7)	11.5 (1.6)
Quinoline	0.04 (0.01)	0.05 (0.00)	0.20 (0.02)	0.16 (0.02)	0.11 (0.01)
Tobacco Specific Nitrosamines (ng/cigarette)					
NAB	1.3 (0.1)	1.9 (0.8)	9.7 (0.8)	8.4 (1.4)	7.6 (0.4)
NAT	5.7 (0.4)	9.1 (2.7)	95.6 (2.4)	98.9 (3.8)	77.6 (0.4)
NNK	10.9 (1.4)	18 (4)	57.6 (4.7)	93.1 (4.1)	66.8 (2.1)
NNN	39.1 (0.2)	47 (6)	113.0 (2.8)	102.8 (2.3)	72.3 (2.8)
Volatiles (µg/cigarette)					
Acrylonitrile	5.6 (0.2)	6.8 (0.8)	10.4 (0.5)	7.5 (0.2)	9.3 (0.1)
Benzene	23 (3)	28 (3)	53 (2)	37 (2)	46 (4)
Butadiene, 1,3-	18 (1)	22 (3)	61 (2)	42 (1)	47 (2)
Isoprene	116 (7)	136 (24)	494 (23)	315 (19)	400 (8)
Styrene	3.6 (0.4)	4.8 (0.1)	24.6 (0.9)	14.6 (0.4)	16.9 (0.9)
Toluene	32 (5)	39 (4)	79 (8)	53 (6)	72 (5)

LOD=level of detection; NAB= N-Nitrosoanabasine; NAT= N-Nitrosoanatabine; NNK= 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN= N-9-nitrosonornicotine

[1] Average analyte values (standard deviations) are based on 6 runs per cigarette brand tested.

The results from this independent, mainstream smoke chemistry analysis of regular and Magic menthol cigarettes demonstrate that combustion of the VLN tobacco variety Vector 21-41 used in the manufacture of X-22 cigarettes produces smoke that is comparable to that produced by conventional, top-selling, commercial cigarette brands currently on the market in the United States. The results also show that the presence of menthol in the filter component of the cigarettes does not significantly affect the chemical composition of the smoke. This provides support that smoke from X-22 cigarettes is not expected to provide any exposure to novel compounds or increase the exposure to a known compound for the subjects in X-22 clinical trials.

6.1.2.2. Arista Laboratories 2004 and 2011 Smoke Chemistry Analyses

A similar smoke composition comparison study was performed by Arista Laboratories for Vector Tobacco on their Quest 3 VLN cigarette made from Vector 21-41 tobacco (the same tobacco used in X-22 cigarettes).[28] The smoke analysis examined Quest 3, other conventional brands, a control reference cigarette (KY2R4F), and a newer lot of reference cigarettes (KY3F4F) for qualitative and quantitative-differences in a spectrum of well-known toxins and carcinogens typically found in combusted cigarettes. This comparison was later updated with the testing of Quest 3 Menthol by the same laboratory.[29] In addition to differing in the presence of menthol, the Quest 3 filter contains an activated carbon segment, while the Quest 3 Menthol filter does not (activated carbon would be expected to absorb menthol). Some analytes were not measured in both sets of analyses, and are indicated by ND (not done) in Table 6-3.

The results from this independent analysis, as shown in Table 6-3, confirm that the combusted smoke from Quest 3 VLN cigarettes is chemically similar to a conventional, American brand (Marlboro Lights), as well as a standard reference cigarette (Kentucky Reference, KY2R4F) tested.

Table 6-3 Mainstream Smoke Chemistry Data[1] Conducted at Arista Laboratories on the Quest 3 Formulation

	Quest 3	**Marlboro Light®**	**KY2R4F**	**Quest 3 Menthol**	**KY3R4F**
Analyte Tested	Project 1808 Aug 2004			Project 11066 Mar 2011	
Tar, Nicotine, CO (mg/cigarette)					
Puffs/Cigarette	6.2 (0.1)	8.3 (0.3)	9.1 (0.2)	5.6 (0.2)	8.3 (0.0)
Tar	7.8 (0.3)	10.8 (0.5)	9.9 (0.6)	11.0 (0.2)	8.4 (0.1)
Nicotine	0.048 (0.003)	0.82 (0.02)	0.81 (0.02)	0.043 (0.001)	0.74 (0.02)
Carbon Monoxide (mg/cigt)	10.1 (0.2)	11.5 (0.7)	12.3 (0.9)	15.1 (1.5)	10.8 (0.4)
Water	0.7 (0.2)	1.0 (0.1)	0.8 (0.2)	2.1 (0.4)	0.79 (0.1)
Ammonia (μ/cigarette	41 (4)	14 (1)	9.6 (0.3)	34 (3)	7.8 (NA)
Aromatic Amines I (ng/ cigarette)					

Analyte Tested	Quest 3	Marlboro Light®	KY2R4F	Quest 3 Menthol	KY3R4F
	Project 1808 Aug 2004			Project 11066 Mar 2011	
Aminobiphenyl, 3-	2.9 (0.2)	2.2 (0.1)	2.0 (0.1)	2.7 (0.2)	1.7 (NA*)
Aminobiphenyl, 4-	2.0 (0.2)	1.5 (0.1)	1.3 (0.1)	1.8 (0.1)	1.1 (NA)
Naphthylamine, 1-	8.1 (0.6)	8.2 (0.4)	7.2 (0.5)	12.3 (0.7)	9.5 (NA)
Naphthylamine, 2-	4.6 (0.3)	5.0 (0.2)	4.6 (0.2)	7.4 (0.5)	6.3 (NA)
Aromatic Amines II (ng/ cigarette)					
Aniline	208 (15)	189 (14)	173 (14)	ND*	ND
Anisidine, o-	2.0 (0.2)	3.0 (0.2)	2.9 (0.3)	ND	ND
Toluidine, m-	40 (3)	38 (3)	38 (6)	ND	ND
Toluidine, o-	38 (3)	39 (3	38 (2)	ND	ND
Carbonyls/Aldehydes (µg/ cigarette)					
Acetaldehyde	345 (24)	619 (52)	656 (69)	786 (20)	758 (NA
Acetone	113 (14)	269 (23)	294 (26)	366 (12)	340 (NA)
Acrolein	21 (3)	66 (7)	66 (8)	53 (2)	56 (NA)
Butryaldehyde	14 (2)	32 (3)	35 (5)	34.4 (0.3)	31 (NA)
Crotonaldehyde	5 (1)	21 (3)	16 (3)	18.9 (0.1)	14 (NA)
Formaldehyde	9.4 (0.9)	25.6 (2.9)	24.9 (3.9)	11.8 (0.2)	14.7 (NA)
Methylethylketone	26 (4)	78 (9)	91 (8)	93 (2)	96 (NA)
Propionaldehyde	20 (2	52 (5)	54 (5)	58 (1)	59 (NA)
Hydrogen Cyanide (µg/cigarette)	77 (3)	127 (8)	111 (7)	307 (16)	120 (NA)
Mercury (ng/ cigarette)	1.34 (0.03)	1.90 (0.07)	2.07 (0.12)	3.12 (0.17)	2.08 (0.14)
Metals (ng/ cigarette)					
Arsenic	2.7 (0.2)	4.3 (0.3)	3.4 (0.2)	3.2 (0.1)	2.7 (0.2)
Cadmium	22 (1)	47 (2)	50 (3)	76 (2)	33 (0.1)
Chromium	<LOD	<LOD	<LOD	0.7 (0.2)	<LOQ (0.5)
Lead	13.3 (0.9)	14.3 (0.8)	12.7 (0.0	18.1 (1.0)	10.4 (0.5)
Nickel	<LOD[2]	<LOD	<LOD	1.4 (0.1)	0.8 (0.1)
Selenium	1.3 (0.1)	2.1 (0.1)	1.0 (0.0)	5.5 (0.2)	2.2 (0.2)
Nitric Oxide (µg/ cigarette)	224 (33)	175 (14)	196 (9)	301 (22)	239 (10)
Phenols (µg/ cigarette)					
Catechol	20 (1)	43 (2)	44 (2)	25 (2)	37 (1)
Cresol, m & p	7.4 (0.5)	6.6 (0.7)	6.5 (0.5)	13.2 (1.2)	6.4 (0.2)
Cresol, o-	2.3 (0.2)	2.2 (0.2)	2.3 (0.2)	4.1 (0.4)	2.3 (0.1)

Analyte Tested	Quest 3	Marlboro Light®	KY2R4F	Quest 3 Menthol	KY3R4F
	Project 1808 Aug 2004			Project 11066 Mar 2011	
Hydroquinone	15 (1)	38 (2)	33 (2)	22 (1)	32 (1)
Phenol	7.9 (0.6)	8.6 (0.8)	7.7 (0.7)	14.9 (1.4)	7.5 (0.3)
Resorcinol	0.34 (0.02)	1.05 (0.05)	0.68 (0.03)	0.61 (0.05)	0.70 (0.02)
Polynuclear Aromatic Hydrocarbons (ng/ cigarette)					
Anthracene	39 (1)	54 (1)	57 (3)	ND	ND
Benz-anthrazene	9.6 (0.4)	14.2 (0.5)	14.1 (0.9)	ND	ND
Benzo[a]pyrene	4.2 (0.2)	6.6 (0.3)	6.2 (0.3)	5.6 (0.3)	5.6 (NA)
Benzo[e]pyrene	3.2 (0.1)	4.2 (0.2)	4.1 (0.3)	ND	ND
Chrysene	9.8 (0.3)	16.3 (0.7)	15.9 (1.0)	ND	ND
Flourene	173 (9)	188 (11)	210 (9)	ND	ND
Flouroanthene	47 (4)	58 (4)	58 (4)	ND	ND
Indeno[1,2,3-cd]Pyrene	2.5 (0.2)	3.7 (0.3)	3.2 (0.4)	ND	ND
Naphthalene	349 (21)	400 (30)	391 (33)	ND	ND
Phenanthrene	122 (6)	152 (8)	161 (12)	ND	ND
Pyrene	28 (2)	44 (2)	43.(2)	ND	ND
Tobacco Specific Nitrosamines (ng/ cigarette)					
NAB	1 (0.1)	16.7 (1.7)	17.0 (0.4)	1.3 (0.2)	15.4 (NA)
NAT	6 (0.5)	128 (10)	132 (8)	8 (0.4)	120 (NA)
NNK	13 (2)	82 (5)	144 (5)	12 (2)	100 (NA)
NNN	79 (2)	138 (6)	148 (4)	83 (4)	111 (NA
Semi-Volatiles (µg/ cigarette)					
Pyridine	3.3 (0.9)	6.0 (0.6)	4.9 (0.4)	11.7 (0.8)	4.7 (NA)
Quinoline	0.22 (0.01)	0.22 (0.02)	0.20 (0.02)	0.39 (0.01)	0.22 (NA)
Vinylpyridine, 3-	0.45 (0.06)	1.47 (0.14)	1.15 (0.05)	ND	ND
Volatiles (µg/ cigarette)					
Acrylonitrile	4.7 (0.3)	8.9 (0.8)	8.5 (1.1)	21.4 (1.3)	9.9 (NA)
Benzene	14 (1)	42 (2)	46 (3)	54 (2)	45 (NA)
Butadiene, 1,3-	19 (2)	40 (4)	39 (2)	49 (3)	42 (NA)
Isoprene	137 (12)	402 (37)	369 (35)	401 (26)	361 (NA)
Styrene	2.7 (0.3)	6.7 (0.7)	6.8 (0.8)	12.4 (0.8)	6.3 (NA)
Toluene	24 (2)	77.(4)	87 (7)	98 (5)	80 (NA)

LOD=level of detection; NAB= N-Nitrosoanabasine; NAT= N-Nitrosoanatabine; NNK= 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN= N-9-nitrosonornicotine

[1] Average analyte values (standard deviations) for Quest 3, Marlboro Light®, and Quest 3 Menthol are based on

	Quest 3	Marlboro Light®	KY2R4F	Quest 3 Menthol	KY3R4F
Analyte Tested	Project 1808 Aug 2004			Project 11066 Mar 2011	
5 or 6 runs per cigarette brand tested in Project 1808 and 3 runs in Project 11066.					

The results from this independent, mainstream smoke chemistry analysis of Quest 3 demonstrate that combustion of the VLN tobacco variety used in the manufacture of X-22 cigarettes produces smoke that is comparable to that produced by combustion of the tobacco used in conventional cigarette brands currently on the market in the United States. This provides support that smoke from X-22 cigarettes is not expected to provide any exposure to novel compounds or significantly increase the exposure to a known compound for the subjects in the proposed clinical trial.

6.2. Pesticide Residues

Although the Environmental Protection Agency (EPA) regulates and specifies which pesticides may be used on tobacco, it does not regulate residues of pesticides approved for use on tobacco in conventional tobacco products. The United States Department of Agriculture (USDA) established standards for imported and domestic tobacco for residues that are not approved for use by the EPA on tobacco.[30] These are specified in 7 CFR §29.427.

Tobaccos to be used in manufacturing X-22 cigarettes for use in the proposed clinical trial comply with standards in 7 CFR §29.427. No changes in agricultural practices were necessary for growing the Vector 21-41 tobacco used in X-22 cigarettes. The low leaf nicotine and total alkaloid content of Vector 21-41 tobacco did not lead to an increased susceptibility to insect pests or diseases when using a typical insect and disease management program. Therefore, existing approved amounts of pesticides labeled for use on tobacco are used to control insect, disease, and weed problems. This conclusion has been supported by the USDA in its determination for the non-regulated status of Vector 21-41 tobacco.[31]

As a result of these controls, under the conditions of usage proposed in the clinical trial, X-22 cigarettes will not pose any additional pesticide exposure risk to subjects in the study beyond those already know to be attributable to smoking standard cigarettes.

7. PHARMACOLOGY AND TOXICOLOGY OF CONVENTIONAL CIGARETTE SMOKE, NICOTINE, AND MENTHOL

The pharmacology and toxicity of tobacco, nicotine, and menthol have been well documented in the literature. Since X-22 cigarettes are comparable to conventional cigarettes on the market, except for the reduction in the level of nicotine and related compounds, the data are briefly reviewed here. The information that follows was obtained from the 2010 Surgeon General's Report[32] and the 2001 Institute of Medicine report, "Clearing the Smoke"[33] and the 2011 Tobacco Products Scientific Advisory Committee's (TPSAC) "Draft Report and Recommendations on Menthol in Cigarettes"[34] unless otherwise noted.

7.1. Pharmacology of Tobacco Smoke and Nicotine

Tobacco smoke consists of thousands of chemical compounds. Several of the compounds are thought to contribute to the pharmacologic activity of tobacco although nicotine, the principal alkaloid in *N. tabacum,* is the largest contributor. Nornicotine and anabasine, other alkaloids in tobacco smoke, have less potent pharmacologic properties than nicotine and are found in much lower levels. Other lesser alkaloids may also influence the activity of nicotine. They both activate the sympathetic nervous system and affect cardiovascular physiology. Additionally, acetaldehyde releases norepinephrine, which results in a pressor effect.

Nicotine is a tertiary amine composed of a pyridine and an N-methylpyrollidine ring. The stereoisomer present in tobacco is (S)-nicotine, which is significantly more pharmacologically active than the synthetic, (L)-nicotine isomer. The level of nicotine in the tobacco plant varies by type, strain, position of leaf on the plant stalk and growing conditions.

The dose-response characteristics of nicotine can be considered biphasic. At low doses such as those received through smoking, nicotine acts on the central nervous system to increase blood pressure and heart rate. At high doses, nicotine acts on the peripheral nervous system, decreasing blood pressure and heart rate. It has been shown that tolerance to tobacco smoke develops after repeated use. First time smokers report nausea, headaches and dizziness that disappear with continued smoking. This response is due largely to the development of tolerance to nicotine.

There is evidence of acute tolerance to some, but not all, actions of nicotine. Very little is known about the mechanism of acute tolerance, although it has been suggested that a nicotine metabolite or the desensitization of nicotine receptors may be involved. Chronic tolerance also occurs for some nicotine effects, shown partially by the fact that smokers increase their tobacco consumption with time. Again, the mechanism is not fully understood, however several hypotheses exist including increased nicotine metabolism or alterations to nicotinic receptors in the brain.

Nicotine has also been shown to improve attention and cognition in some cases. An article reviewed studies that demonstrated the benefits nicotine administration may have on several disease states.[35] Nicotine was shown to lower the incidence of symptoms and diagnosis of Alzheimer's disease (AD). AD patients who were administered nicotine showed improvement in attention and a reduction in errors on learning tasks. Nicotine administration to patients suffering from Parkinson's disease (PD) has resulted in improvement in attention and motor skills. Schizophrenic patients also have benefited from nicotine, noted by improvements in spatial perception, memory and attention. In people diagnosed with attention deficit hyperactivity disorder (ADHD), nicotine has improved concentration and reaction time.

Constituents in tobacco inhibit both forms of the enzyme monoamine oxidase (MAO-A and MAO-B). This enzyme is important in the breakdown of the amine neurotransmitters, including dopamine, which is thought to mediate the reinforcing effects of nicotine and contribute to tobacco dependence.[36]

7.2. Pharmacokinetics of Nicotine in Cigarettes

Nicotine is carried on tar droplets that are inhaled by a smoker. The pH of tobacco smoke impacts the absorption of nicotine in the mouth since the free base is more readily absorbed. Once tobacco smoke reaches the small airways and alveoli in the lungs, nicotine is absorbed rapidly. Two reasons account for this: the large surface area within this part of the body and the alkaline pH (approximately 7.4). Once absorbed in the lungs, nicotine passes into the blood stream and is distributed throughout the body. The spleen, lungs, liver and brain all have high affinities for nicotine, which has a distribution half-life of approximately 9 minutes.[37]

Nicotine is metabolized largely by the liver, although metabolism in the lungs also occurs. The two principal metabolites of nicotine are cotinine and nicotine-N'-oxide while some free nicotine is also excreted in the urine. Cotinine is formed in the liver first by the oxidation of position 5 of the pyrrolidine ring as mediated by cytochrome P-450 to nicotine *D1*'(5')-iminium ion.[38] This ion is then metabolized in a second step by cytoplasmic aldehyde oxidase to cotinine.[39] Nicotine-N'-oxide is formed by the oxidation of the nitrogen atom of the pyrrolidine ring.[40] The excretion of nicotine from the kidney occurs through both glomerular filtration and tubular secretion and is dependent upon the pH of urine.

7.3. Toxicology of Tobacco Smoke and Nicotine

As discussed previously, tobacco smoke exerts a wide range of pathologies. The Surgeon General recently reported that smoking causes, or contributes to, many cancers including: lung, oral, laryngeal, esophageal, bladder, cervical, pancreatic, stomach, kidney cancer and leukemia.[32] Smoking causes respiratory diseases including: chronic obstructive pulmonary disease, pneumonia, respiratory effects in utero and respiratory effects in childhood, adolescence and adulthood. Smoking has been linked to cardiovascular diseases with respect

to causal relationships found in association with the following conditions: subclinical atherosclerosis, coronary heart disease, cerebrovascular disease and abdominal aortic aneurysm. Additionally, smoking causes reproductive and other effects including: fetal deaths and still birth, fertility, low birth weight, pregnancy complications, cataracts, diminished health status/morbidity, hip fractures, low bone density and peptic ulcer disease.

The 2010 Surgeon General Report[32] also reviewed the mechanism by which the toxicity of tobacco smoke affects the lungs. Generally, the gas phase components of smoke penetrate the small airways and alveoli and may play a role in diseases such as emphysema while the particulate phase components are deposited in the larger airways and play a role in diseases such as chronic bronchitis. Smoking over time causes a narrowing of the smaller airways. Cigarette smoke has been shown to increase epithelial permeability in all areas of the lungs, which decreases its protective function. Furthermore, smoke can increase the respiratory tract secretions and/or decrease ciliary activity, thereby decreasing the protective action of the mucociliary system in the lung.

The acute toxicity of nicotine brings about dizziness, nausea, vomiting, headache and diarrhea. More severe intoxication can occur, resulting in death, due to paralysis of respiratory muscles. Green tobacco sickness is an occupational illness due to the dermal absorption of nicotine present in the dew on tobacco leaves. The symptoms are similar to the toxic effects of inhaled nicotine.

While it is difficult to assess the direct contribution of nicotine to tobacco-related effects due to the complex mixture of chemicals in tobacco smoke, several observations have been made. Chronic exposure to nicotine in tobacco smoke affects many body systems and contributes to the progression of several diseases. It may cause atherosclerotic disease by acting on lipid metabolism and coagulation, by hemodynamic effects and/or by endothelial injury. Nicotine contributes to acute coronary events by causing increased oxygen demand, thrombosis or coronary spasm.

Furthermore, nicotine may contribute to the progression of chronic hypertension by aggravating vasoconstriction. Chronic nicotine exposure reduces skin blood flow which may slow wound healing time. Nicotine use during pregnancy increases the risk of low birth weight, prematurity, spontaneous abortion, and perinatal mortality in humans. The development of emphysema may be influenced by nicotine. Finally, nicotine is not genotoxic.

7.4. Nonclinical Toxicology of Menthol in Cigarettes

The Tobacco Products Scientific Advisory Committee of the Center for Tobacco Products of the Food and Drug Administration was mandated to produce a report on the public health impact of menthol in cigarettes. The resulting "Draft Report and Recommendations on Menthol in Cigarettes"[34] was made public in April of 2011. The information contained in this section was taken from that report.

As detailed in Chapter 7 of the TPSAC report, some information on the understanding of the differential risks posed by menthol versus non-menthol cigarettes comes from toxicological studies. The relevant studies include *in vitro* and *in vivo* approaches using menthol or smoke from cigarettes. As for toxicologic studies in general, there are questions about the relevance of animal and cell toxicology studies with respect to the potential toxic effects of menthol in cigarette smokers.

Various studies have addressed the toxicity of menthol using *in vitro* cellular assays that assess whether menthol damages or kills cells. One general issue in interpreting such studies is the relevance of the concentrations used and the endpoints investigated to toxicity in smokers. One study (Bernson and Pettersson, 1983, as cited in the TPSAC report) investigated the toxicity of menthol in four different bioassays. They summarize the findings as suggesting that menthol may lead to "…a deterioration of biological membranes." Other studies have shown that menthol affects cell membrane properties. Azzi *et al*. (2006, as cited in the TPSAC report) used a system that measures diffusion of carcinogens across porcine esophageal tissue to assess the effect of menthol on permeation and reservoir formation in the tissue for NNK and B[a]P. Menthol slowed the diffusion of these two carcinogens but increased the size of the tissue reservoir for NNK. In another cell system, menthol increased the transepithelial electrical resistance, but the decrease was not different from that observed with non-menthol cigarettes (Alakayak and Knall 2008, as cited in the TPSAC report).

Several studies summarized in the TPSAC report have addressed interactions of menthol with membrane receptors. Sidell *et al*. (1990, as cited in the TPSAC report) used a human neuroblastoma cell line and identified a calcium channel that was blocked by menthol. More recent studies have focused on the TRPM8 Ca2+-permeable channel. Results from various cellular systems show that activation of the TRPM8 channel by menthol induces cell death (Yamamura *et al*. 2008; Li *et al*. 2009, as cited in the TPSAC report), although one study using prostate cancer cells found that TRPM8 activation was not the mechanism of menthol-induced cell death in that system (Kim *et al*. 2009, as cited in the TPSAC report).

Another inhalation study was reported in the peer-reviewed literature in 1997 (Gaworski *et al*. 1997, as cited in the TPSAC report). In this study, Fischer 344 rats were exposed to mainstream smoke from a reference cigarette and a similar cigarette containing 5000 ppm synthetic *l*-menthol. The only difference noted between the two exposure groups was a dose-response trend with level of particulate matter for nasal discharge in the reference cigarette group but not in the menthol cigarette group.

A report on a study conducted by Reynolds Tobacco Co. of a 90-day inhalation study comparing menthol vs no menthol in heated tobacco vs. conventional cigarettes (Salgado and Glantz 2011, as cited in the TPSAC report) indicated that menthol inhalation from heated tobacco produced more severe histopathological changes in the lungs compared to conventional cigarettes.

CONCLUSIONS

The TPSAC report reached the following conclusions regarding menthol, based on the evidence reviewed:

- The evidence is insufficient to conclude that it is more likely than not that menthol cigarette smokers inhale more smoke than non-menthol cigarette smokers. Because of methodologic issues in studying smoking topography, the generalizability of these finding to the smoking of menthol cigarettes in daily life is questionable.

- The evidence is insufficient to conclude that it is more likely than not that menthol cigarette smokers are exposed to higher levels of nicotine and other tobacco smoke toxins, at least in regular daily smokers of more than 5 or 10 cigarettes per day. There are insufficient data to know if menthol cigarettes result in greater smoke intake and more exposure to tobacco smoke toxins among smokers of relatively few cigarettes per day.

- The evidence is insufficient to conclude that smokers of menthol cigarettes face a different risk of tobacco-caused diseases than smokers of non-menthol cigarettes. Some toxicology studies raise concern, particularly the finding that the addition of menthol is associated with greater fine particles which are suspected to contribute to cardiovascular disease. Available epidemiologic data do not demonstrate increased disease risk in people, but the data are largely limited to lung cancer. The hypothesis that menthol cigarette smoking increases the risk of cardiovascular disease is biologically plausible and needs to be investigated.

8. SAFETY ASSESSMENT OF VERY LOW NICOTINE TOBACCO, THE ACTIVE INGREDIENT IN X-22 CIGARETTES

Cigarettes, including X-22 cigarettes, cause many serious and life-threatening conditions such as cancer, cardiovascular disease, respiratory disease and reproductive effects.[41] Furthermore, X-22 is intended for smoking following combustion, as opposed to ingestion. The toxicity of cigarette smoke, in terms of disease outcomes, is well understood and is not differentiated by brand nor reduced by changes in tar and nicotine yields. The HHS reported in 2001 that changes in cigarette design, including concomitant reductions in tar and nicotine yields, from the 1950s to the mid-l980s had no convincing effect on the disease burden caused by cigarette use, either for smokers as a group or the entire population. Thus, any change in nicotine content, a minor component of tar yield, as a result of the incorporation of VLN tobacco in X-22 cigarettes is not expected to be clinically relevant. Cigarettes should be considered maximally toxic, in that the thousands of compounds in tobacco smoke may make X-22 cigarettes as toxic and harmful to human subjects as continuing to smoke their usual brand. However, it should be noted, unlike conventional cigarettes sold over-the-counter, X-22 cigarettes used in clinical trials with confirmed smokers or prescribed by a physician to confirmed smokers, will be used for a relatively short time period and for smoking cessation.

The genetic modification in Vector 21-41 involves the specific reduction of expression of an endogenous nicotine biosynthesis enzyme in the roots and the insertion of the selectable marker gene, *npt II*. The *nptII* gene was the first selectable marker for plant transformation to be approved for human consumption.[42] The biosafety of the use of kanamycin resistance as a selectable marker in transgenic plants has been discussed extensively.[43,44] The only new protein possibly expected in Vector 21-41 is *npt-II* protein. The *npt-II* protein has no significant homology with proteins listed as food allergens or toxins and is instable to proteolytic enzymes, heat, and stomach acid conditions. For these reasons, allergenicity or toxicity were not a concern.[45] Tobacco is not consumed as a food, but rather smoked. Therefore like other proteins in tobacco, *npt-II* would be broken down during the curing, aging, processing, and burning of the tobacco prior to human exposure.[46] An enzyme-linked immunosorbent assay (ELISA) test could not detect any *npt-II* protein in cured, processed Vector 21-41 tobacco leaf. Breakdown products and derivatives of proteins in tobacco Vector 21-41 would be similar to those derived from other proteins normally found in non-transgenic tobacco leaves to which humans are exposed.

8.1. Nonclinical Studies of Vector 21-41 Tobacco

Studies of the toxicological effects of the products of combustion of cigarettes made with VLN tobacco from Vector 21-41 has been done by Chen *et al*.[47] A material termed cigarette smoke condensate (CSC), collected by filtering smoke from two types of cigarettes made with Vector 21-41 tobacco (Quest 1 and Quest 3) and KY2R4F cigarettes, was subjected to a

battery of *in vitro* tests to determine its toxicological profile. CSC from Quest 1 (nicotine yield 0.53 mg/cigarette) and Quest 3 (nicotine yield 0.032 mg/cigarette) had less nicotine than CSC from a standard reference cigarette, KY2R4F (nicotine yield 0.80 mg/cigarette). The authors reported that in addition to nicotine, some tobacco-specific nitrosamines (TSNAs), aldehydes, and volatile organic compounds were also reduced in the Quest cigarettes compared to the KY2R4F. Aromatic amines were slightly lower in CSC from Quest 1 and slightly higher in CSC from Quest 3.

CSC from all three types of cigarettes was positive for mutagenic potential in the Ames test, produced a similar concentration-dependent increase in lactate dehydrogenase in the cell culture medium of normal human bronchial epithelial cells, and increased cytoplasmic nucleosomes, the sub-G1/G0 population of cells, and the frequency of apoptotic comet tails. CSC from either of the Quest cigarettes inhibited cell growth more than CSC from the reference cigarette, and adding nicotine to the CSC from Quest cigarettes attenuated this inhibition. CSC from Quest cigarettes also decreased gap junction intercellular communication, caused cell cycle arrest, and increased proapoptotic protein Bax independent of p53 induction. The authors concluded that nicotine attenuates cytotoxicity possibly in part through inhibition of apoptotic pathways.

A study by Cantanzaro *et al*[48] studied the effect of mainstream cigarette smoke from Quest 1, Quest 3, and KY2R4F cigarettes on juvenile apolipoprotein E-deficient mice exposed for 8 and 12 weeks. In both studies, mice exposed to smoke from all three cigarettes developed greater areas of lipid-rich aortic lesions than did non-smoking controls. Smoke from the very-low-nicotine Quest 3 resulted in smaller aortic lesions than did smoke from Quest 1 or KY2R4F cigarettes. Urinary levels of isoprostane F2 alpha VI, increased proportionally to cigarette nicotine yield, whereas induction of pulmonary cytochrome P4501A1 was proportional to tar yield. Lesion area was associated with both nicotine and tar yields, although in multiple regression analysis only nicotine was a significant predictor of lesion area. Smoke exposure did not alter systolic blood pressure (SBP), heart rate (HR), blood cholesterol, or leukocyte count. Taken together, these observations suggest that smoking may accelerate atherosclerosis and that nicotine may be one of the causative factors.

A study was performed to explore the effects of varying TSNA levels in low nicotine tobacco products.[49] TSNAs are important carcinogens in smokeless tobacco products and cigarette smoke. Two of these tobacco alkaloid derived compounds, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N9-nitrosonornicotine (NNN), are consistently carcinogenic in laboratory animals, with NNK showing higher activity. TSNA levels were lower in very-low-nicotine Quest 3 cigarettes than in conventional cigarettes, although NNN was only slightly reduced (0.83 μg/g for Quest 3 vs 2.8 μg/g for Marlboro Light).

Ramachandran *et al*[50] studied the effect of mainstream and sidestream cigarette smoke extracts from ordinary high-nicotine cigarettes (Marlboro 100's), low-nicotine cigarettes (Quest 1), and VLN cigarettes (Quest 3) on the thrombogenic properties of platelets under flow conditions that approximately mimicked normal vascular blood flow. Extracts from low- and VLN cigarettes caused significantly greater platelet activation than extracts from

higher-nicotine cigarettes. That nicotine was crucial was confirmed by adding 50 nM nicotine (comparable with the nicotine level in smokers' plasma) to VLN cigarette smoke extracts and demonstrating a reduction in the shear-dependent rate of platelet activation of more than 75%. The authors conclude that although low- and very low nicotine cigarettes may be useful in weaning smokers from cigarettes, they also might cause a significantly increased risk of thrombotic disease in smokers and others exposed to the secondhand smoke from such cigarettes. This hypothesis can be tested only in controlled clinical trials, which is one reason that 22nd Century is proposing to develop X-22 as a prescription drug, rather than as an over the counter product.

A second study[51] employed a hemodynamic shearing device (HSD) to investigate the *in vitro* effects of cigarette smoke on platelet activation and endothelial cell adhesion of the activation in a combined system of platelets and cultured human umbilical vein endothelial cells under physiological shear stress. The authors investigated the alteration of E-selectin expression on endothelial cells upon exposure to: (1) platelets and Quest 3 ("nicotine-free") smoke extract, (2) platelets alone, and (3) Quest 3 smoke extract alone, under physiological shear stress. They confirmed that nicotine decreases platelet activation due to cigarette smoke and found that:

(i) surface expression of E-selectin on endothelial cells was significantly increased upon simultaneous exposure of endothelial cells and platelets to Quest 3 extracts relative to exposure of endothelial cells to either platelets or Quest 3 smoke extract alone ($p<0.05$),

(ii) platelet activation was significantly increased in the presence of Quest 3 smoke extract ($p<0.05$), and

(iii) nicotine (200 nM) when added to Quest 3 smoke extract, significantly reduced platelet activation due to Quest 3 smoke extract ($p<0.05$), an effect additionally confirmed by conventional cigarette extracts which contain nicotine ($p<0.05$).

It was concluded that: (a) "nicotine-free" smoke extract and platelets additively increase endothelial cell E-selectin surface expression and (b) nicotine modulates platelet activation regardless of endothelial cells.

9. CONCLUSIONS BASED ON NONCLINICAL DATA

9.1. Safety of the Usage Levels in the Proposed Clinical Trial

In the first clinical trial, X-22 cigarettes will be provided in a limited amount for 6 weeks only to confirmed smokers. As described in Section 10.3.4, when smoking VLN cigarettes, smokers generally consume fewer cigarettes per day than when they smoke their usual brand. It is expected that the exposure of subjects to smoke components in the trial will, in general, be no greater than if they did not participate and continued to smoke their usual brand.

22nd Century believes that the product, as designed and manufactured, is not more or less harmful than conventional cigarettes when used as directed in this clinical trial.

9.2. Conclusions Regarding Human Safety

Cigarette smoking is the cause of many serious and life-threatening conditions. The United States Surgeon General has reported that smoking causes, or contributes to, many cancers including: lung, oral, laryngeal, esophageal, bladder, cervical, pancreatic, stomach, kidney cancer and leukemia. Smoking causes respiratory diseases including: chronic obstructive pulmonary disease, pneumonia, respiratory effects in utero and respiratory effects in childhood, adolescence and adulthood. Smoking has been linked to cardiovascular diseases with respect to causal relationships found in association with the following conditions: subclinical atherosclerosis, coronary heart disease, cerebrovascular disease and abdominal aortic aneurysm. Additionally, smoking causes reproductive and other effects including: fetal deaths and still birth, fertility, low birth weight, pregnancy complications, cataracts, diminished health status/morbidity, hip fractures, low bone density and peptic ulcer disease.

Results of the smoke composition comparison study show that the smoke from X-22 cigarettes contains no novel toxic compound(s) and that human subjects who inhale smoke from X-22 cigarettes will not be exposed to greater amounts of known toxic compound(s) than when they inhale smoke from standard US cigarette brands.

The addition of menthol to the X-22 cigarette is not expected to have any impact of the relative toxicity of the cigarette smoke, based on the following conclusions reached by the TPSAC in their draft report:

> The evidence is insufficient to conclude that smokers of menthol cigarettes face a different risk of tobacco-caused diseases than smokers of non-menthol cigarettes. Some toxicology studies raise concern, particularly the finding that the addition of menthol is associated with greater fine particles which are suspected to contribute to cardiovascular disease. Available epidemiologic data do not demonstrate increased disease risk in people, but the data are largely limited to lung cancer. The hypothesis that menthol cigarette smoking increases the risk of cardiovascular disease is biologically plausible and needs to be investigated.

VLN cigarettes, including X-22 cigarettes, have been shown in nonclinical studies to demonstrate similar toxicological effects to standard cigarettes. Relatively recent nonclinical studies suggest that nicotine attenuates tobacco smoke-related cytotoxicity (possibly in part through inhibition of apoptotic pathways), that nicotine may be one factor that accelerates the development of tobacco smoke-related atherosclerosis, and that nicotine attenuates (but does not eliminate) the increased risk of tobacco smoke-related thrombotic disease. The fact that nicotine contributes in various ways to numerous tobacco smoke-related health risks is one reason that 22nd Century is proposing to develop X-22 as a prescription drug.

Based on this body of information, it is reasonable to conclude that X-22 cigarettes pose no new hazard or health risk beyond the risks known for conventional cigarettes and that, under the conditions of usage proposed in the clinical trial, X-22 cigarettes should not pose any additional risk to subjects in the study beyond those already know to be attributable to smoking conventional cigarettes.

10. CLINICAL STUDIES

10.1. Overview

This section will provide background information on studies conducted using VLN cigarettes made with Vector 21-41 tobacco and provide a rationale for the use of X-22 cigarettes for smoking cessation in human subjects.

As discussed in Section 5.1, cigarettes containing very low levels of nicotine have been manufactured in the past. These "denicotinized" cigarettes differ from cigarettes such as X 22 cigarettes in that they are manufactured with conventional tobacco from which nicotine has been removed by chemical processes. Denicotinized cigarettes included Next® manufactured by Philip Morris Inc. and a line of reduced and low nicotine cigarettes manufactured by Ultratech/Lifetech Corporation. Next® cigarettes had nicotine removed using a high pressure carbon dioxide process and Ultratech/Lifetech cigarettes had nicotine removed with a mild alkaline (ammonium solution) wash. Processes involved in removing nicotine from tobacco have been found not be specific for nicotine and other components are also removed, resulting in changes in taste and other factors.[24] Levels of monoamine oxidase inhibitors (MAOIs) such as β-carboline alkaloids, naphthoquinone, and acetaldehyde are also affected by extracting nicotine from tobacco.

In contrast to denicotinized tobacco, the tobacco used in X-22 cigarettes is produced from a very low nicotine (VLN) tobacco variety that when grown to maturity using conventional practices contains approximately 95% less nicotine than conventional tobacco plants. X-22 cigarettes may therefore offer benefits relative to cigarettes manufactured with conventional tobacco that has had nicotine chemically removed.

Quest cigarettes, manufactured using the same VLN tobacco as X-22 cigarettes, were sold by Vector Tobacco Inc. from December 2002 to December 2007, and Quest Menthol cigarettes were sold by Vector Tobacco Inc. from November 2003 through December 2007. Consumers purchased these Quest and Quest Menthol cigarettes for at least another year. Both Quest and Quest Menthol cigarettes were produced in brand styles containing 3 levels of nicotine in the tobacco filler, resulting in the following nicotine yields per cigarette: Quest 1 (0.60 milligrams [mg]), Quest 2 (0.3 mg) and Quest 3 (≤0.05 mg). Quest 1 and Quest 2 were made with mixtures of regular tobacco and Vector 21-41 to produced reduced nicotine levels. Quest 3 and Quest 3 menthol were manufactured only with Vector 21-41 tobacco and are therefore most similar to X-22 Smoking Cessation Product.

According to filings by Vector Group Ltd. (parent company of Vector Tobacco Inc.) with the Securities and Exchange Commission, sales of Quest by Vector Tobacco Inc. from 2002 to 2007 were approximately $60 million. This equates to approximately 26 million packs (520 million cigarettes) sold or 14,246 pack-a-day smokers using Quest for 5 years. Since Quest was sold by Vector Tobacco in only 8 U.S. states (New York, New Jersey,

Pennsylvania, Ohio, Indiana, Illinois, Michigan, and Arizona) representing 31% of U.S. smokers, approximately 1.4 million smokers tried Quest. Assuming the trial rate was even among Quest 1, Quest 2, and Quest 3 (which was labeled "nicotine free" on the packaging), approximately 467,000 smokers used Quest 3 VLN cigarettes. Furthermore, in published studies reviewed within this section, nearly 1000 subjects were exposed to VLN cigarettes containing Vector 21-41 tobacco under controlled conditions.

Although studies of denicotinized cigarettes have been published, VLN X-22 cigarettes are very different from cigarettes made with conventional tobacco from which nicotine was chemically removed. Therefore, only relevant literature conducted using Quest cigarettes is summarized below.

10.2. Previous Clinical Studies Using Very Low Nicotine Cigarettes

10.2.1. Studies of Smoking Cessation

The following studies were primarily conducted to evaluate the use of VLN cigarettes for smoking cessation.

10.2.1.1. A Study of Quest 1, Quest 2, and Quest 3 in a Step-down Treatment Schedule With and Without Nicotine Replacement Compared to a Conventional Cigarette and the Influence of Genetics on Treatment Success

A randomized double-blind, active controlled, parallel group, multi-center phase II clinical trial was reported by Becker *et al* to evaluate the efficacy of reduced-nicotine cigarettes as a novel smoking cessation treatment under IND 69,185.[53,54]

Treatment consisted of 6 weeks of smoking a series of cigarettes with progressively lower nicotine content (Quest 1, Quest 2, and Quest 3, with smoking yields of 0.6, 0.3 and ≤0.05 mg nicotine/cigarette), either in combination with nicotine patch therapy (NRT) or placebo patches. Three hundred forty-six smokers of non-menthol cigarettes who were motivated to quit were randomized to one of 3 treatments:

- Group 1: Quest plus NRT patch,
- Group 2: Quest plus placebo patch, or
- Group 3: active control (conventional cigarette) plus NRT patch.

The design of the study is presented in Table 10-1, below

Table 10-1 Design of Study Published by Becker *et al*, 2008

	Weeks																			
								Primary endpoint 4-week Abstinence												
Group	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	----	32
1	Usual Brand all groups	Quest 1		Quest 2		Quest 3													3-month post-quit follow-up		6 month post-quit follow-up
						21 mg patch		21 mg patch				14 mg patch		7 mg patch		placebo patch					
2		Quest 1		Quest 2		Quest 3															
						placebo patch		placebo patch				placebo patch		placebo patch		placebo patch					
3		Active Control		Active Control		Active Control															
						placebo patch		21 mg patch				21 mg patch		14 mg patch		7 mg patch					

The primary endpoint was 4 weeks of continuous abstinence (Weeks 7 to 10), with follow-up at 3 and 6 months. Group 1 had a higher continuous abstinence during weeks 7-10 than Group 3, 32.8% vs. 21.9% ($p = 0.04$). Group 2 had an abstinence rate similar to that of Group 3, 16.4% vs. 21.9% ($p = 0.89$). Abstinence rates at the 3-month follow-up were 17.2%, 10.3%, and 14.9% in Groups 1, 2, and 3, respectively. At the 6-month follow-up, abstinence rates were 11.2%, 7.8%, 9.6% in Groups 1, 2, and 3, respectively. The differences in abstinence among groups were not statistically significant at 3 months and 6 months. Mean exhaled CO levels increased moderately compared to baseline after subjects smoked Quest 1 (Group 1, +1.5 parts per million [ppm]; Group 2, +2.5 ppm) and Quest 2 (Group1, +2.9 ppm; Group 2, +2.9 ppm), and was reduced compared to baseline after smoking Quest 3 (Group 1, -5.4 ppm; Group 2, -3.0 ppm). No serious adverse events (SAEs) were attributed to the investigational product.

10.2.1.2. A Study of Quest 3 or Quest 2 Compared to Nicotine Lozenge

Another controlled cessation phase II trial conducted by Hatsukami *et al*[55] compared the effects of smoking VLN cigarettes or low nicotine cigarettes to the use of nicotine lozenges. Smokers were randomly assigned to 1 of 3 treatment groups which were each administered for 6 weeks:

- Group 1: Quest 3 cigarettes having a nicotine yield ≤0.05 mg (n=53)
- Group 2: Quest 2 cigarettes having a nicotine yield 0.30 mg (n=52)
- Group 3: 4 mg nicotine lozenge (n=60)

Four week continuous abstinence in the Hatsukami trial was measured after the treatment period, as shown in Figure 10-1 below, rather than during the last four weeks of the treatment period. (According to the prescription Chantix® label, 4-week continuous abstinence in the Chantix® phase III clinical trials was measured during the last 4 weeks of the 12-week treatment period, while patients were still taking Chantix®.)

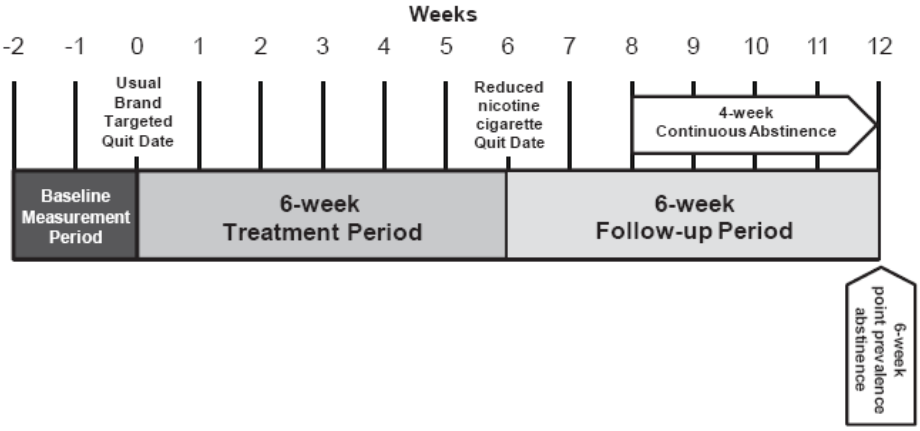


Figure 10-1 Design of Smoking Cessation Study Published by Hatsukami *et al*, 2010

Continuous abstinence 4-weeks post-treatment was 43% for the VLN cigarette group, 35% for the nicotine lozenge group, and 21% for the low nicotine cigarette group. Point abstinence rates at 6 weeks post-treatment were 47% for the VLN cigarette group, 37% for the nicotine lozenge group, and 23% for the low nicotine group. Unlike the Quest 2 cigarette, the Quest 3 VLN cigarette was not associated with compensatory smoking behaviors. Furthermore, Quest 3 VLN cigarette and nicotine lozenge were associated with reduced carcinogen exposure, nicotine dependence and product withdrawal scores. The Quest 3 VLN cigarette was associated with greater relief of withdrawal from usual brand cigarettes than the nicotine lozenge. The Quest 3 VLN mg cigarette led to a significantly higher rate of cessation than the Quest 2 0.3 mg nicotine cigarette and a similar rate as nicotine lozenge.

Patients who used the VLN Quest 3 cigarette over the 6-week treatment period significantly reduced their smoking as compared to their usual brand of cigarettes. The number of VLN cigarettes smoked per day on average decreased from 19 (the baseline number of cigarettes of the smoker's usual brand) to 12 by the end of the 6-week treatment period, even though participants in this clinical trial were instructed to smoke *ad libitum* during treatment. As depicted in Figure 10-2 below, carbon monoxide (CO) levels, an indicator of smoke exposure, significantly decreased from 20 ppm (baseline) to 15 ppm. Cotinine, a metabolite and biomarker of nicotine, significantly decreased from 4.2 micrograms/milliliter (mL) (baseline) to 0.2 micrograms/mL. All differences in the above three measurements were statistically significant ($p<0.05$).

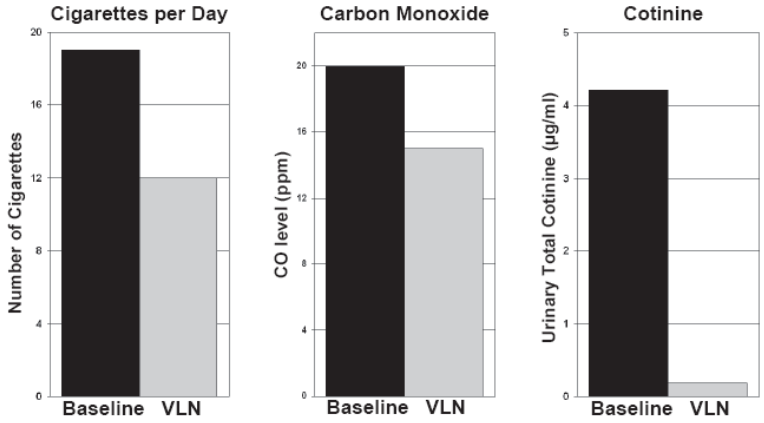


Figure created by 22nd Century from data published by Hatsukami *et al*, 2010

Figure 10-2 Number of Cigarettes Smoked, Carbon Monoxide, and Cotinine Results Published by Hatsukami *et al*, 2010

Additional biomarkers of smoke exposure were significantly reduced on average from baseline measurements (taken before the 6-week treatment period) in patients who used the Quest 3 VLN cigarette (see Figure 10-3).

BIOMARKER	DESCRIPTION	LEVEL IN PATIENTS (pmol/mg creatinine)		REDUCTION
		Baseline	VLN Cigarette	
NNAL	Metabolites of the tobacco-specific carcinogen NNK	0.92	0.2	78%
NNN	Metabolites of the tobacco-specific carcinogen NNN	0.09	0.03	67%
1-HOP	Metabolite of pyrene, a marker for uptake of carcinogenic polycyclic aromatic hydrocarbons	0.89	0.57	36%
3-HPMA	Metabolite of the smoke toxicant acrolein	3320	1453	56%
S-PMA	Metabolite of the carcinogen benzene	2.46	0.76	69%

All differences were statistically significant (P<0.05).

Figure created by 22nd Century from data published by Hatsukami *et al*, 2010

Figure 10-3 Biomarkers Measured after Smoking Quest 3 Cigarettes by Hatsukami *et al*, 2010

No significant differences were observed in biomarkers for cardiovascular risk between groups.

In a separate publication, Drgon *et al*[56] reported genome wide association (GWA) results from participants in the above clinical trial. GWA studies have previously demonstrated haplotypes that distinguish successful quitters from individuals who were not able to quit smoking using smoking cessation methods other than VLN cigarettes.[57,58] In this study, DNA extracted from the blood of self-reported European-American subjects was pooled into: i) three pools (n = 16 in each) containing DNA from "successful quitters"; and ii) 6 pools of "unsuccessful quitters" who were selected based on matches to features noted for the successful quitters. When the authors compared data from unsuccessful versus successful quitters, there was significant clustering of nominally positive single nucleotide polymorphisms (SNPs) in small chromosomal regions. The authors concluded that these data support the idea that ability to quit in a circumstance in which most participants used VLN cigarettes shares strong overall genetic influences with ability to quit when other pharmacological strategies were used.

10.2.1.3. A Comparison of Quest 1 Without Nicotine Replacement Therapy Versus Quest 3 With Nicotine Replacement Therapy for Cessation Success

In a randomized, controlled clinical trial conducted at Roswell Park Cancer Institute in Buffalo, New York, Rezaishiraz *et al*[59] investigated the effect of smoking a VLN cigarette in combination with a nicotine patch for 2 weeks prior to the quit date. Ninety-eight adult heavy smokers (using 20 or more cigarettes/day) were randomized to 1 of 2 treatments:

- Group 1: 2 weeks of Quest 3, nicotine yield ≤0.05 mg, and 21-mg nicotine patch before the quit date; or
- Group 2: Quest 1, nicotine yield 0.6 mg, during the 2 weeks before the quit date.

After the quit date, all subjects received counseling for smoking cessation and nicotine patch therapy for up to 8 weeks (4 weeks of 21-mg patches, 2 weeks of 14-mg patches, and 2 weeks of 7-mg patches). Group 1, which used VLN cigarettes and nicotine patch before quitting had lower combined craving score during the 2 weeks before ($p = 0.001$) and after ($p = 0.013$) the quit date. Self-reported point prevalence of smoking abstinence at the 3- and 6-month follow-ups was higher in Group 1 (43% vs. 34% and 28% vs. 21%, respectively), although the difference was not statistically significant at the $p < 0.05$ level.

10.2.1.4. Study of Quest 3 With or Without Nicotine Replacement Therapy or With Quest 1 and Quest 2 in a Step-down Treatment Schedule

A small study was carried out by Jed Rose *et al*[60] at Duke University comparing the following groups:

- Group 1: Quest 3 (nicotine yield ≤0.05 mg, tar yield 9 mg) with NRT
- Group 2: Quest 3 without NRT

- Group 3: Use of a series of cigarettes of decreasing nicotine content and the following nicotine yields: Quest 1, 0.6 mg; Quest 2, 0.3 mg; and Quest 3, ≤0.05 mg

During the first week of the study, subjects smoked only their usual brand (UB) of cigarette. From Weeks 2 through 5, subjects gradually reduced the amount of usual brand and increased the amount of Quest 3 smoked (Groups 1 and 2), or smoked cigarettes with decreasing amounts of nicotine per cigarette (Group 3). During Weeks 6 through 11, subjects were to smoke Quest 3 exclusively. During Week 12 through Week 14, subjects were gradually weaned off Quest 3, and subjects using NRT were weaned off of nicotine replacement. In Group 2, 33% of subjects achieved 4-week continuous abstinence. Group 3 attained a 13% abstinence rate. In Group 1 no subjects achieved abstinence.

10.2.2. Other Studies of Quest 3 Cigarettes

The following studies evaluated Quest 3 cigarettes but did not directly research their use as a tool for smoking cessation.

10.2.2.1. A Study of the Effects of Inhaled Nicotine, Very Low Nicotine Cigarettes, and Regular Cigarettes on Craving, Withdrawal, Self-Administration in Male and Female Smokers

This study by Barett[61] examined the acute subjective effects of nicotine in the absence of tobacco (through nicotine inhalers and placebo inhalers), tobacco with greatly reduced nicotine (Quest 3 cigarettes, nicotine yield ≤0.05 mg), and nicotine-containing tobacco (Quest 1 cigarettes, nicotine yield 0.60 mg), and their effects on subsequent smoking behavior. A total of 22 smokers (12 male, 10 female; 11 low dependent, 11 high dependent) were enrolled. During 4 randomized blinded sessions, participants self-administered inhaled nicotine, inhaled placebo, Quest 1 cigarettes or Quest 3 VLN cigarettes and assessed their effects using Visual Analogue Scales and the Brief Questionnaire of Smoking Urges. They could then self-administer their preferred brand of cigarettes using a progressive ratio task. Quest 1 cigarettes and Quest 3 cigarettes were each associated with increased satisfaction and relaxation as well as decreased craving relative to the inhalers and Quest 1 cigarettes increased ratings of stimulation relative to each of the other products. Both Quest 1 and Quest 3 cigarettes delayed the onset of preferred tobacco self-administration relative to inhaled nicotine and inhaled placebo, but only Quest 1 cigarettes reduced the total amount self-administered. Sex differences were evident in the effects of VLN cigarettes on withdrawal-related cravings with women experiencing greater VLN cigarette-induced craving relief than men. The authors concluded that these findings suggest that VLN tobacco is effective in acutely reducing many smoking abstinence symptoms, especially in women, but a combination of nicotine and non-nicotine tobacco ingredients may be necessary to suppress smoking behavior.

10.2.2.2. An Evaluation of Affect Relief Produced by Smoking

Perkins *et al*[62] conducted a study to determine if smoking relieved negative affect only due to smoking abstinence or also from other sources. Dependent smokers (n=104) randomly assigned to one of three smoking conditions (Quest 1, nicotine yield 0.60 mg, Quest 3, nicotine yield ≤0.05 mg, or no smoking) completed four negative mood induction procedures (one per session): 1) overnight smoking abstinence, 2) challenging computer task, 3) public speech preparation, and 4) watching negative mood slides. A fifth session involved a neutral mood control. The 2 smoking groups took 4 puffs on their assigned cigarette and then smoked those same cigarettes *ad libitum* during continued mood induction. All subjects rated their level of negative affect and positive affect on several measures and also craving and withdrawal.

Results indicated that negative affect relief from smoking depended on the negative affect source (i.e., mood induction procedure) and the affect measure. Smoking robustly relieved negative affect due to abstinence on all 4 measures but only modestly relieved negative affect due to the other sources and typically on only some measures. Smoking's effects on positive affect and withdrawal were similar to effects on negative affect, but relief of craving depended less on negative affect source. Smoking reinforcement only partly matched the pattern of negative affect relief. Few responses differed between the Quest 1 and VLN Quest 3 smoking groups. The authors concluded that acute negative affect relief from smoking depends on the situation and the affect measure used but may not depend on nicotine intake.

10.2.2.3. A Comparison of the Acute Effects of Potential Reduced-Exposure Products Marketed to Smokers

This study by Cobb *et al*[63] compared the acute effects of various noncombustible potential reduced exposure products (PREPs) (Ariva compressed tobacco tablet, Camel Snus, Marlboro Snus, and Commit® 2 mg nicotine lozenge) and various combustible tobacco products (Subjects' OWN brand, SHAM [puffing on an unlit cigarette], and Quest 3 [nicotine yield ≤0.05 mg]) on plasma nicotine levels, expired CO, heart rate, and subjective effects in 28 participants during 7 laboratory sessions (one session for each product with 2 administration periods per product). Subjects abstained from smoking overnight before each session.

Nicotine: On average, 5 minutes after beginning to smoke a single OWN brand cigarette, participants' mean plasma nicotine level increased by approximately 18 nanograms (ng)/ml. Non-combustible PREPs included in this study delivered a nicotine dose an order of magnitude less, and any significant increases were observed later, if at all. Neither Quest 3 nor SHAM increased plasma nicotine level reliably.

CO: Expired CO increased to a greater extent in the OWN condition compared to Quest 3 after the first administration (OWN, mean 12.8 ppm (standard error of the mean [SEM] 0.7); Quest 3, 11.0 ppm [SEM 0.6]) and second product administration (OWN, mean 17.5 ppm

[SEM 1.0]; Quest 3, 14.4 ppm [SEM 0.8]). There were no significant changes in any other condition.

Heart Rate: For OWN, mean baseline heart rate was 67.8 beats per minute (bpm) (SEM 1.9), which increased significantly to 82.3 bpm (SEM 2.3) 5 minutes after the first product administration and remained significantly elevated. Significant increases relative to baseline also were observed after the second administration. For Quest 3, mean baseline heart rate was 68.7 bpm (SEM 2.1), which increased significantly but to a lesser extent than OWN to 73.1 bpm (SEM 2.1) 5 minutes after the first but not the second product administration. Mean heart rate in the SHAM condition was stable or decreased over both administration periods. For the non-combustible conditions, a significant increase in heart rate was observed during the Camel Snus condition; heart rate at baseline was 67.8 bpm (SEM 2.3), which increased significantly to 72.0 bpm (SEM 2.4) 15 minutes after the first product administration. No significant increases were observed for ARIVA, COMMIT® or Marlboro Snus.

Subjective Effects: OWN brand was associated with significant decreases in craving scores relative to baseline at nearly every time point. Quest 3 was associated with a similar pattern, while mean craving scores during SHAM were relatively high and stable. During all non-combustible conditions, craving scores were significantly higher relative to OWN at 5 and 15 minutes after the first product administration and at 5, 15, 30 and 45 minutes after the second product administration

10.2.2.4. Evaluation of the Effect of Puff Volume and Compensatory Smoking on Changes in Filter Tar Staining in Quest Cigarettes

Strasser *et al*[64] conducted a study to describe the physical characteristics of the Quest cigarettes, to evaluate the effect of puff volume on filter tar staining, and to examine the effect of compensatory smoking on changes in filter tar staining. In this study, 50 adult smokers smoked four 4 cigarettes in a laboratory setting. The first cigarette was always their own preferred brand cigarette followed by Quest 1 (nicotine yield 0.6 mg), Quest 2 (nicotine yield 0.3 mg), and Quest 3 (nicotine yield ≤0.05 mg) cigarettes. Nicotine level was blinded to researcher and participant, and order of presentation was randomized and counterbalanced across participants to minimize order effects. All cigarettes were smoked 30 minutes apart. Spent cigarette filters were analyzed digitally. Results suggest that compensatory smoking may be detectable by darkening and reddening of the tar stain. The authors concluded that this type of measure could be useful in quantifying the extent to which human smokers smoke differently than standard testing protocols and in assessing the prevalence of compensatory smoking in population samples.

10.2.2.5. Evaluation of Compensatory Smoking with Reduced Nicotine Cigarettes

Strasser *et al*[65] also conducted a study to test the hypothesis that compensatory smoking will occur as nicotine levels of cigarettes decrease, increasing exposure to tobacco toxins using a

smoking topography machine. In this study, 50 adult smokers participated in a single laboratory session. Subjects first smoked one of their own brand cigarette through the device. Thirty minutes after smoking their own brand cigarette, participants smoked the first of three Quest cigarettes (Quest 1 [nicotine yield 0.6 mg], Quest 2 [nicotine yield 0.3 mg] or Quest 3 [nicotine yield ≤0.05 mg]) with a 30-minute interval between cigarettes. The Quest cigarettes were presented in randomized order, counter-balanced across participants to minimize potential order effects. Subjects and researcher were blind to nicotine level. Puff volume was measured using the smoking device. Prior to and 4 minutes after smoking each cigarette, participants provided breath CO samples. Upon extinguishing each cigarette, participants completed a 14-item subjective rating of the cigarette they had just finished smoking, including which had the most and least amount of nicotine.

Mean total puff volumes measured were 832.0 ml, 540.3 ml, 518.1 ml, and 570.5 ml in the Own brand, Quest 1, Quest 2, and Quest 3 cigarette groups. Mean number of puffs per cigarette were 14.3, 9.8, 9.9, and 10.0 in the Own brand, Quest 1, Quest 2, and Quest 3 cigarette groups. Fifty-four percent correctly identified the Quest 1 cigarette as the highest nicotine cigarette. Fifty-eight percent correctly identified the Quest 3 cigarette as the least nicotine-containing cigarette. Only 38% correctly identified both the Quest 1 cigarette and Quest 3 cigarette. Ability to discriminate nicotine levels did not affect total puff volume or CO boost.

10.2.2.6. Inpatient Study of Reduced Nicotine Cigarettes, Very Low Nicotine Cigarettes, or No Cigarettes over 11 Days

Smoking behavior with reduced nicotine cigarettes (RNCs) and VLN cigarettes under controlled, inpatient, conditions over 11 days was studied by Donny *et al*.[66] Thirty adult regular smokers were assigned randomly to 1 of 3 groups:

- Group 1: RNCs (Quest 1, nicotine yield 0.6 mg),
- Group 2: VLN cigarettes (Quest 3, nicotine yield ≤0.05 mg), or
- Group 3: No smoking.

Subjects in the VLN cigarette group smoked fewer cigarettes (a decrease of 3.8 cigarettes/day compared to baseline vs. an increase of 2.1 cigarettes/day in the RNC group), took fewer puffs per cigarette (8.8 vs. 11.5, $p = 0.10$), tended to leave larger butts (0.54 grams vs. 0.45 grams, $p = 0.11$), and had significantly lower expired CO levels. Subjects smoking VLN cigarettes also demonstrated a 31% decline in the number of puffs earned on a progressive ratio, a measure of the motivation to smoke, during the study. Acute craving suppression following smoking was evident throughout the study regardless of nicotine content.

10.2.2.7. Modulation of Smoking-Induced Platelet Activation by Nicotine

In a previous *in vitro* study, Girdhar *et al*[51] exposed human blood cell platelets and cultured endothelial cells to smoke extracts from nicotine-containing cigarettes and VLN cigarettes, and found that nicotine has a protective effect on platelet activation induced by exposure to cigarette smoke. In this *in vivo* study, Girdhar *et al*[94] reported that RNCs increase platelet activation in smokers. In this study, 32 smokers smoked 3 cigarettes with moderately reduced nicotine (Quest 1, nicotine yield 0.6 mg) in 1 hour. Following the first 1 hour of smoking, 16 of these subjects continued smoking with 5 of the same cigarettes over the next 1.5 hours, while the other 16 subjects smoked 5 VLN cigarettes (Quest 3, nicotine yield ≤0.05 mg). (Consuming 8 cigarettes over 2.5 hours equates to approximately 51 cigarettes/16-hour day and 5 VLN cigarettes over 1.5 hours equates to approximately 53 cigarettes/16-hour day.) The percentage of platelets scored as activated increased in both groups, but the increase was larger in the group smoking VLN cigarettes during the second period ($p < 0.02$). No measure of smoke exposure was made, so it cannot be determined whether nicotine content of the cigarettes affected the intake of smoke under the rapid smoking schedule.

To determine whether nicotine modulates platelet activation by secondhand smoke, 16 nonsmokers were exposed to medium-nicotine smoke and 16 to VLN smoke for 1.5 hours on two consecutive days. Exposure to second hand smoke increased platelet activation by 60%, but no difference between the medium and VLN groups was observed ($p>0.09$). The authors concluded that in smokers, nicotine modulates platelet activation. See further discussion of this study in Section 10.4.1.3.4.

10.2.2.8. The Influence of Instructions and Nicotine Dose on Subjective and Reinforcing Effects of Smoking

Perkins *et al*[67] conducted a study in 2004 to examine the subjective and reinforcing effects of smoking. In this study, 96 healthy smokers whose preferred brand had a nicotine yield of at least 0.7 mg were assigned to 1 of 4 treatment groups and evaluated during a single laboratory session. Participants smoked one of their preferred brand cigarettes *ad libitum* upon arrival and then rested quietly for 1 hour. At the end of the 1-hour rest period, they completed baseline measures of craving and withdrawal. They then were introduced to the designated cigarette (regular nicotine or low nicotine) and were given the following instructions, depending on their group assignment:

- Told regular nicotine: "We are interested in why smokers like certain brands of cigarettes. Regular Full-Flavor brands, those higher in nicotine, often are liked. So, nicotine levels may have something to do with brand liking. Today, you will be smoking a regular higher nicotine cigarette that is well-liked."

- Told low nicotine: "We are interested in why smokers do not like certain brands of cigarettes. Low nicotine brands often are not liked, so nicotine levels may have something to do with brand liking. Today, you will be smoking a low nicotine cigarette that is not well-liked."

Subjects rated the cigarette according to "how much nicotine" it included and completed craving and withdrawal measures.

In this study, instructions about the nicotine content and expected likeability of a cigarette influenced ratings of the cigarette on items relevant to the perceived nicotine content and strength of the cigarette, as well as on some measures of smoking reward. The greater ratings for the VLN cigarette among those told regular nicotine versus those told low nicotine support a significant placebo effect. Similarly, the lower ratings for the regular nicotine cigarette among those told low nicotine versus those told regular nicotine support a significant antiplacebo effect. Instructions had comparable effects regardless of the nicotine dose of the cigarette. Under the conditions of this study, instructions did not affect craving or withdrawal. Women showed similar responses to instructions compared with men, but results indicated greater effects due to nicotine on ratings regarding perception in women compared with men.

10.2.2.9. The Influence of the Airway Sensory Effect of Nicotine on the Reward Obtained from Cigarette Puffs

Naqvi and Bechara[68] examined the extent to which reward from puffs can be derived from the airway sensory effect of nicotine, in the absence of a direct central nervous system effect of nicotine. To do this, the self-reported reward obtained from individual puffs from Quest 1 (nicotine yield 0.6 mg), VLN Quest 3 (nicotine yield ≤0.05 mg), and unlit cigarettes within 7 seconds of inhalation, which is before nicotine had an opportunity to reach the brain, was assessed. Results indicated that Quest 1 puffs were rated as both stronger and more rewarding than VLN and unlit puffs. Furthermore, the extent to which nicotine elicited reward was directly correlated with the extent to which nicotine elicited airway sensations. The authors concluded that the airway sensory effects of nicotine contribute to the reward from puffs, above and beyond the reward derived from the airway sensory effects of nonnicotine constituents.

10.2.2.10. Evaluation of Naltrexone or Bupropion on the Reinforcing Value of Nicotine

Rukstallis *et al*[69] evaluated the effect of naltrexone or bupropion on the self-reported reinforcing effects of nicotine. This study explored the effects of an acute dose of naltrexone, bupropion, or placebo on the relative reinforcing value of nicotine from cigarette smoking using Quest 1 (nicotine yield 0.6 mg) and VLN Quest 3 (nicotine yield ≤0.05 mg) cigarettes. In a double-blind, within-subjects design, 26 dependent smokers participated in 3 experimental cigarette smoking sessions following pretreatment with either naltrexone

(50 mg), bupropion (300 mg), or placebo. After medication administration and 2 hours of monitored deprivation from cigarettes and food, participants rated their responses to the initial exposure to the cigarettes and then participated in 4 choice sessions over a 2-hour period during which they could take 4 puffs from either cigarette. The relative reinforcing value of nicotine, as measured by the number of Quest 1 puffs chosen out of 16, was significantly lower following naltrexone compared to placebo. There were no effects of an acute dose of bupropion on cigarette choices. The authors concluded that naltrexone may reduce the relative reinforcing effects of nicotine via cigarette smoking and support ongoing investigation of opioid antagonists as potential smoking cessation pharmacotherapies.

10.2.2.11. Evaluation of Gender Differences in the Effect of Dose Instructions on the Reinforcing Effects of Nicotine

Perkins *et al*[70] conducted a study in 2006 comparing the influence of the presence versus absence of dose instructions on the subjective and reinforcing effects of nicotine via cigarette smoking in men and women. A total of 120 subjects were randomly assigned to 1 of 4 groups. Subjects abstained overnight from smoking. Half of the subjects received Quest 1 (nicotine yield 0.6 mg), and the other half received VLN Quest 3 (nicotine yield ≤0.05 mg) cigarettes. Furthermore, half of each subsample was accurately instructed they were receiving a "normal nicotine" or a "no nicotine" cigarette, while the other half received no instructions. Subjects completed baseline measures of craving and mood (positive and negative affect), took 2 puffs from the cigarette after receiving dose instructions or no instructions, and then rated the cigarette's "reward" value (liking, satisfying) and other characteristics. They also repeated the craving and mood measures. Subjects then smoked more of that same brand *ad libitum* over the next 30 minutes to measure reinforcement (puff number and latency to first puff). Overall, nicotine increased reward, other cigarette ratings, and positive affect, but did not affect craving or smoking behavior. However, results varied by sex. Dose instructions enhanced the effects of nicotine on smoking reward and reinforcement in women, while instructions tended to dampen or even reverse these effects of nicotine in men. The authors concluded that in women but not in men, the influence of nicotine on smoking reward and reinforcement is enhanced by accurate verbal information about the cigarette's nicotine dose.

10.2.2.12. A Study of the Effect of Genetic Variants of the Mu Opioid Receptor on the Reinforcing Effects of Nicotine

Noting the importance of the endogenous opioid system in nicotine reward, Ray *et al*[71] conducted a study to elucidate the possible role of genetic variation in the mu opioid receptor (MOR) in smoking behavior. Specifically, this study examined (1) the association between polymorphisms of the gene encoding the MOR (ie, gene OPRM1; specifically, SNP 118A to G substitution) with the relative reinforcing value of nicotine using a validated nicotine cigarette choice paradigm and (2) the influence of the MOR antagonist naltrexone on nicotine reinforcement. A total of 156 subjects were screened and genotyped at the A118G SNP to obtain a final sample of 60 eligible subjects; 30 with the AA genotype and 30 with either the AG genotype (26 subjects) or GG genotype (4 subjects). In a within-subject, double-blind

design, subjects participated in 2 experimental sessions following 4 days of orally administered naltrexone 50 mg or placebo. Participants completed a validated assessment of the relative reinforcing value of nicotine. This cigarette choice paradigm assessed self-administration of Quest 1 (nicotine yield 0.6 mg) vs. Quest 3 (nicotine yield ≤0.05 mg) cigarettes after a brief period of nicotine abstinence. The relative reinforcing value of nicotine (number of nicotine cigarette puffs) was predicted by a significant OPRM1 by gender interaction. Among women, the low activity G allele (A/G and G/G) was associated with a reduced reinforcing value of nicotine; among male smokers, there was no association with genotype. Smokers carrying a G allele were also significantly less likely to differentiate the Quest 1 vs. Quest 3 cigarettes by subjective ratings of satisfaction and strength. No evidence for an effect of naltrexone on nicotine reinforcement was found in the overall sample or in the genotype or gender subgroups.

10.2.2.13. A Study of the Expectancy of Nicotine and the Pharmacological Effects of Nicotine on Memory and Subjective Effects of Cigarettes

In this double-blind, 2-hour, laboratory study, Kelemen and Kaighobadi evaluated the expectancy and pharmacological effects of nicotine on memory and the subjective effects of cigarettes[72] A total of 120 smokers who had abstained from nicotine for 8 hours prior to the study were randomly assigned to one of the following 4 groups:

- Told nicotine - Received nicotine (Quest 1, nicotine yield 0.60 mg/cigarette)
- Told nicotine - Received no nicotine (Quest 3, nicotine yield ≤0.05 mg/cigarette)
- Told no nicotine - Received nicotine (Quest 1, nicotine yield 0.60 mg/cigarette)
- Told no nicotine - Received no nicotine (Quest 3, nicotine yield ≤0.05 mg/cigarette)

Each subject was informed of which kind of cigarette they would receive as shown above, then smoked 1 entire cigarette, and then rated the cigarette on a number of dimensions and completed questionnaires on smoking urges, tension, and energy. Participants also completed tests of memory as well as predictions of memory. Pharmacology played a stronger role than expectancy in most ratings of the cigarettes, but significant effects of expectancy did emerge for feelings of increased wakefulness, concentration, calming, cigarette satisfaction, and hunger reduction. The higher level of nicotine significantly reduced smoking urges, but expectancy alone reduced tension after smoking. Neither variable produced significant effects on memory or memory predictions.

10.2.2.14. The Acute Effects of Smoking on the Brain as Measured by Functional Magnetic Resonance Imaging

Lindsey *et al*[73] studied the acute effects of smoking on the brain using functional magnetic resonance imaging (fMRI) during tobacco cigarette and placebo tobacco smoking. Subjects smoked either a Marlboro Red brand cigarette or a VLN Quest 3 cigarette during fMRI

scanning with concurrent assessment of heart rate and changes in subjective mood state. Visual analog scales for mood states were presented every 2.5 minutes throughout the functional scan. Using General Linear Modeling techniques, changes in subjective sensation of "high" after smoking tobacco were found to be most highly correlated with blood oxygen level dependence (BOLD) signal change in the bilateral anterior nucleus of the thalamus, bilateral caudate head and tail, bilateral posterior cingulate (these and all subsequent correlations were found to be significant at $p<0.0001$). These findings are consistent with both the reinforcing properties of nicotine and the anatomical distribution of terminals emanating from regions of high nicotinic acetylcholine receptor density. Changes in subjective sensation of "high" after smoking placebo tobacco were most highly correlated with BOLD signal change in the orbitofrontal cortex, and dorsolateral prefrontal cortex. The authors noted that these findings are consistent with the cognitive components of reward mediated by conditioned reinforcers only in the absence of active drug.

10.2.2.15. The Effect of an Extinction-based Smoking Cessation Treatment (Quest 3 plus Nicotine Patch) on Brain Responses to Smoking Cues

McClernon *et al*[74] reported that the combination of smoking VLN cigarettes and a nicotine patch has been reported to extinguish brain responses to smoking cues. In this study, blood oxygen level-dependent functional magnetic resonance imaging of 16 dependent smokers were scanned at baseline, after 2-4 weeks of smoking Quest 3 nicotine yield ≤0.05 mg cigarettes while wearing a 21-mg nicotine patch, and 2-4 weeks following quitting smoking altogether. The treatment attenuated responses to smoking cues in the amygdala while potentiating responses to control cues. Exploratory analysis indicated that this pattern was also observed in the thalamus of future abstinent but not relapsing smokers. The results of this preliminary study suggest that an extinction-based treatment for smoking cessation alters brain responses to smoking and control cues in amygdala.

10.2.2.16. Pharmacological, Stimulus Expectancy, and Response Expectancy Effects of Nicotine-Free Cigarettes

Kelemen[75] conducted a study to evaluate the pharmacological, stimulus expectancy, and response expectancy effects of Quest 1 (nicotine yield 0.60 mg) compared with Quest 3 (nicotine yield ≤0.05 mg). In this double-blind study, 103 smokers who had abstained from smoking for at least 5 hours were told that they would be given a cigarette to smoke and that the cigarette may or may not have nicotine in it. Subjects were offered an additional $5 if they could guess whether the cigarette contained nicotine after smoking it. Subjects completed a series of pre-tests (baseline measurement of smoking urges, baseline measure of energy and tension, and baseline assessment of cognitive performance) and then smoked a single cigarette (Quest 1 or Quest 3). After smoking, subjects guessed whether or not their cigarette contained nicotine and predicted how it would influence their subsequent cognitive performance. The also repeated the baseline questionnaires and a second round of cognitive testing.

Cigarettes with nicotine were preferred on most dimensions, and stimulus expectancy partially mediated the relationship between nicotine and subjective effects of the cigarettes. Stimulus expectancy also mediated the effect of nicotine on tension reduction immediately after smoking, but not 30 minutes later. Response expectancy effects of nicotine were related to predicted and actual recall performance, such that participants who performed well tended to attribute the effect to the cigarette they smoked.

10.2.2.17. A Study of the Effects of Nicotine on Ratings of Attractiveness

Attwood *et al*[76] conducted this double-blind study to test the hypothesis that nicotine enhances the hedonic impact of behaviors performed in the presence of nicotine, using ratings of facial attractiveness. Male (n=24) and female (n=24) participants attended a single testing session and were randomized to smoke either Quest 1 (nicotine yield 0.60 mg) or Quest 3 (nicotine yield ≤0.05 mg) cigarette, after which they completed ratings of attractiveness of 20 male and 20 female faces. A 2 × 2 × 2 mixed-model repeated measures analysis of variance indicated a significant main effect of cigarette, reflecting higher attractiveness ratings in the Quest 1 compared with the Quest 3 condition There was no evidence that these effects differed between males and females. There were also no effects on subjective ratings of mood, suggesting that the effects observed on ratings of attractiveness may not simply reflect global hedonic effects, or a positivity bias in questionnaire responding, in the nicotine condition.

10.2.2.18. Effect of Nicotine on Smoking-dependent Dopamine Release

Brody *et al*[77] studied the effect of dopamine release in response to cigarette smoking and nicotine gum administration. This study used VLN cigarettes and ^{11}C-raclopride positron emission tomography (PET) scanning to investigate whether nicotine is necessary for smoking-induced dopamine release. A total of 62 subjects were enrolled, and an MRI scan of the brain was obtained within 1 week of the study PET sessions. Subjects were instructed to smoke one of their usual brand cigarettes immediately prior to testing. They then were interviewed, filled out rating scales, and underwent a session of bolus-plus-continuous-infusion ^{11}C-raclopride PET scanning for evaluation of ventral striatal ^{11}C-raclopride binding potential, an indirect measure of dopamine release in this area of the brain. Three hours after their previous cigarette, subjects took a 10-minute break from the PET scan during which they smoked either a regular (the subject's own brand, de-identified) or VLN Quest 3 (nicotine yield ≤ 0.05 mg) cigarette under double-blind conditions. Rating scales evaluating craving, anxiety, and mood were administered immediately before and after smoking.

Smoking a regular cigarette resulted in a significantly greater mean reduction in ^{11}C-raclopride binding potential in the ventral striatum than smoking a VLN cigarette. Although both groups had reductions in craving and anxiety with smoking, the regular cigarette group had a greater improvement in mood. For the total group, change in binding potential correlated inversely with change in mood, indicating that greater smoking-induced dopamine release was associated with more smoking-related mood improvement. Thus, nicotine delivered through cigarette smoking appears to be important for ventral striatal dopamine

release. Study findings also suggest that mood improvement from smoking is specifically related to ventral striatal dopamine release.

10.2.2.19. Effect of Nicotine Inhalation on Nicotinic Acetylcholine Receptor Occupancy in the Brain

Brody *et al*[78] conducted this study in follow-up to a previous report that smoking regular cigarettes resulted in 88% occupancy of $\alpha_4\beta_2$ brain nicotinic acetylcholine receptors (nAChRs). This study investigated whether nicotine is solely responsible for the previously observed receptor occupancy, or whether other factors related to smoking are involved. The authors hypothesized that if nicotine was solely responsible for the receptor occupancy, then smoking a cigarette without nicotine should decrease receptor binding to within the range of 22% to 24%. To investigate this hypothesis, 24 PET sessions were performed on tobacco-dependent smokers, using 2-[F-18]fluoro-A-85380 (2-FA), a radiotracer that binds to $\alpha_4\beta_2$ nAChRs. 2-FA displacement was determined from before to 3.1 hours after either: no smoking, smoking a VLN cigarette (Quest 3, nicotine yield ≤0.05 mg), or smoking a reduced nicotine cigarette (Quest 1, nicotine yield 0.60 mg). Analysis of this PET data revealed that smoking a VLN and a low-nicotine cigarette resulted in 26% and 79% $\alpha_4\beta_2$ nAChR occupancies, respectively, across three regions of interest. Therefore, the authors concluded that nicotine inhalation during smoking appears to be solely responsible for $\alpha_4\beta_2$ nAChR occupancy.

10.2.2.20. A Study of the Effects of Nicotine and Gender on Alcohol-induced Increases in Smoking Behavior

Alcohol is known to increase smoking urges and smoking behavior. To evaluate if nicotine content of cigarettes or gender of subjects influences this effect, King *et al*[79] enrolled male and female smokers who were non-dependent heavy social drinkers in 2 double-blind laboratory sessions. Subjects were randomized to receive either an alcohol (n=29) or placebo (n=13) beverage and then smoked either VLN Quest 3 (nicotine yield ≤0.05 mg), or Quest 1 (nicotine yield 0.60 mg) cigarettes over a 3-hour period. Results showed that alcohol, compared with placebo beverage, increased both men's and women's smoking urge, as well as subjective ratings of smoking reference puffs for either Quest 1 or VLN cigarettes. Regardless of cigarette type, alcohol (more than placebo) increased men's smoking behavior, including puff count, volume, and duration. In contrast, for women, smoking topography measures did not differ between alcohol and placebo conditions. The authors concluded that the results indicate that the mechanisms underlying co-use of alcohol and tobacco in women may be more complex than in men.

10.2.2.21. Evaluation of Silver Acetate Interactions with Nicotine and Non-nicotine Smoke Components

Oral topical silver-containing formulations were marketed in the 1970s and 1980s as smoking deterrents, based on the finding that when using such formulations, an unpleasant taste occurs

upon smoking. This study by Rose *et al*[80] explored the basic mechanistic question of whether topical oral silver acetate solution interacts with nicotine as opposed to non-nicotine smoke constituents. A total of 20 smoking volunteers were recruited to rate higher nicotine (Usual brand or Quest 1, nicotine yield 0.60 mg) or VLN (Quest 3, nicotine yield ≤0.05 mg) cigarettes, as well as the Nicotrol® nicotine vapor inhaler and sham (air) puffs. In 2 sessions, subjects rated the sensory and hedonic qualities of puffs after rinsing their mouths with either silver acetate solution or deionized water (placebo). Silver acetate relative to placebo solution substantially reduced liking and satisfaction ratings for the usual brand and VLN cigarettes; in contrast, for the nicotine inhaler these ratings were unaffected by the silver-based treatment. These results support the conclusion that silver acetate not only renders the taste of cigarette smoke less appealing, but also that the compound appears to interact selectively with non-nicotine smoke constituents. Moreover, these data suggest silver acetate would be compatible with buccal nicotine delivery systems (e.g., nicotine lozenge or gum).

10.2.3. Ongoing Clinical Trials Registered on ClinicalTrials.gov

There are currently 2 clinical trials recruiting subjects for smoking cessation studies using VLN cigarettes that are registered on ClinicalTrials.gov. The first is NCT01050569, entitled "Innovative Interventions for Smoking Cessation", principal investigator Dorothy Hatsukami, PhD at the University of Minnesota. VLN cigarettes containing Vector 21-41 and designed by 22nd Century are being used in this phase II clinical trial currently in progress. The Hatsukami study plans to enroll approximately 200 subjects to evaluate the effect of a combination of the 21 mg nicotine patch and a VLN cigarette compared to the 21 mg nicotine patch only and VLN cigarette only on abstinence and withdrawal symptoms.

The second is NCT00960778, entitled "Gender Differences in Response to Nicotine Replacement Therapy and 'Denicotinized Cigarettes' (BIRCWH)", principal investigator Karen Hartwell, MD at the Medical University of South Carolina. The Hartwell study plans to enroll 20 subjects to investigate the underlying neurobiology of differences between male and female smokers. Quest 3 VLN cigarettes and the nicotine patch will be utilized in the study.

10.3. Clinical Efficacy of X-22 for Smoking Cessation

10.3.1. Overview

Although numerous studies of cigarettes containing VLN tobacco have been published (see Section 10.2), only a handful provide meaningful evaluations of the efficacy of these cigarettes as a smoking cessation tool. Data are available in a clinical study report from one phase II clinical study and in publication format from 5 others. Data from these studies is discussed below. Carbon Monoxide results are discussed in the summary of safety information (Section 10.4).

10.3.2. Efficacy Results from Vector-201 Phase II Clinical Trial

The efficacy of cigarettes manufactured with VLN tobacco was systematically evaluated in one IND clinical trial: Vector-201, A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of Quest Alone and in Combination with Nicotine Replacement Therapy as a Smoking Cessation Aid.[53,54]

As summarized in Sections 10.2.1.1 and 10.4.1.2, a total of 346 healthy smokers who were motivated to quit smoking were enrolled in this double-blind trial and followed over 8 months, and 85 subjects completed the study. Subjects were randomized to one of the following groups:

- Group 1: Quest 1, Quest 2, and Quest 3 plus NRT patch
- Group 2: Quest 1, Quest 2, and Quest 3 plus placebo patch
- Group 3: Conventional active control cigarettes plus NRT patch

A diagram showing the study design is provided in Figure 10-4. During the first 6 weeks (Week 1 to Week 6), subjects in Groups 1 and 2 smoked *ad libitum* for 2 weeks at each nicotine level beginning with Quest 1, then proceeding to Quest 2, and finally to Quest 3. Subjects in Group 3 received a blinded control cigarette with a conventional nicotine content. At Week 5, Quest use was supplemented in Group 1 with the addition of a transdermal nicotine patch (21 mg), while subjects in Groups 2 and 3 added a placebo patch for 1 week. Visit 5 (end of Week 6/beginning of Week 7) was the "quit date".

In Group 1, during weeks 7 to 10, subjects continued to receive a 21 mg transdermal nicotine patch for an additional 4 weeks, followed by a 14 mg nicotine patch for 2 weeks (Weeks 11 and 12), and 7 mg nicotine patch for 2 weeks (Weeks 13 and 14), and a placebo patch for 2 weeks (Weeks 15 and 16).

In Group 2, during Weeks 7 to 16, subjects continued to receive a placebo patch.

In Group 3, subjects continued to use NRT on the standard of care schedule: i.e., subjects received a 21 mg transdermal nicotine patch for 6 weeks (Weeks 7 to 12), followed by a 14 mg nicotine patch for 2 weeks (Weeks 13 and 14), and a 7 mg nicotine patch for 2 weeks (Weeks 15 and 16).

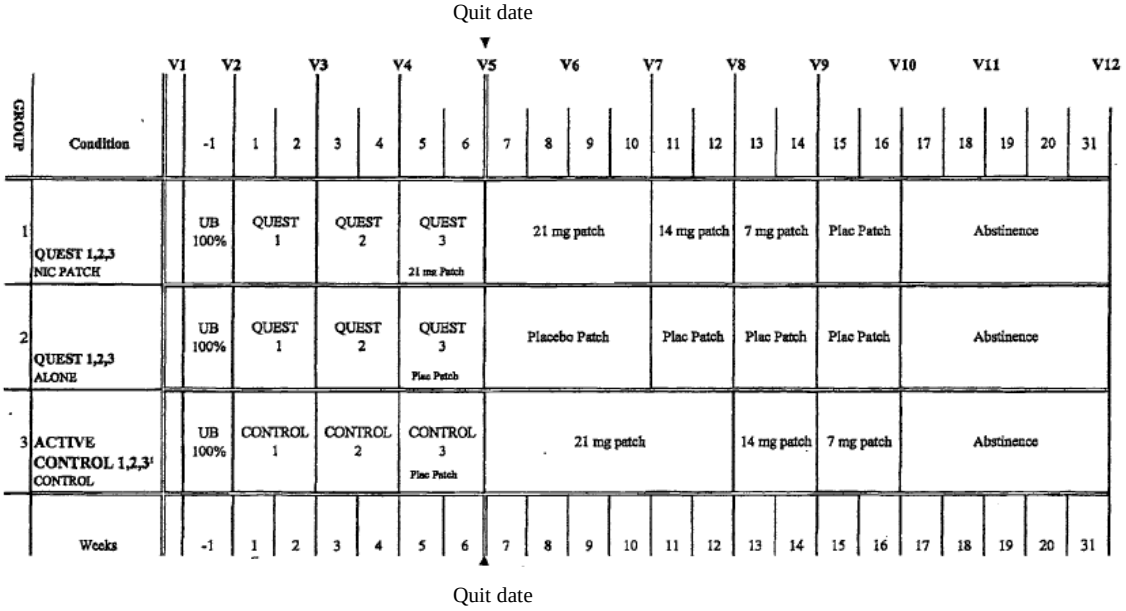


Figure 10-4 Design of Study Vector-201

10.3.2.1. Primary Efficacy Endpoint in Study Vector-201: 4 Week Abstinence Rates

The primary efficacy endpoint was 4 weeks of continuous abstinence from smoking. The protocol required that subjects quit smoking at Visit 7 (Week 7), and 4-week abstinence was determined at Visit 7 (Week 11). Abstinence was determined by self report and verified by exhaled CO less than 10 ppm. If at least one of these 2 conditions was not met, the subject was classified as having failed to meet the primary endpoint.

The primary analysis was done on the intent-to-treat (ITT) population using all available data. Subsequently, the ITT population was analyzed using statistical methodology to impute missing values. All analysis methods and results are provided in the clinical study report;[54] a summary is provided in Table 10-2, below.

Table 10-2 Summary of Four Week Abstinence Results by Method of Imputation from Study Vector-201

Analysis/Imputation Method	Group 1 Proportion (p-value[a])	Group 2 Proportion (p-value[a])	Group 3 Proportion
Based on Available Data (ITT Population)[b]	43.2%	24.1%	26.3%
Primary Analysis: Using Treatment Failure (ITT Population)	32.8% (0.0499)	16.4% (0.8922)	21.9%
Using Treatment Failure for Subjects who Discontinued Due to Lack of Efficacy (Excluding Subjects Whose Abstinence Status Could Not be Determined)	41.8% (0.0169)	22.9% (0.7455)	26.0%
Using Multiple Imputation (ITT Population)	44.0% (0.0297)	29.3% (0.4567)	28.6%
Using Treatment Failure (Per Protocol Population)	45.5% (0.0213)	20.0% (0.9017)	27.8%

[a] One-sided Fisher's exact test for 4-week abstinence proportions compared with Group 3, at the 0.05 and 0.25 levels using Bonferroni adjustment.
[b] For the available population (ITT), statistical significance was not computed due to diminished sample size.
Source: CSR for Study Vector-201, in-text Table 12, Four-Week Abstinence Proportions by Method of Imputation

For the available population (ITT), statistical analysis was not computed due to the diminished sample size. Using all other statistical analysis methods, this study showed that Group 1 was statistically different from Group 3, under a 0.05 significance level. Using the Bonferroni adjusted value (i.e., 0.025), statistical significance was maintained in the ITT Treatment Failure analysis for subjects who discontinued due to lack of efficacy. Irrespective of the analysis, no significant differences were noted when comparing Group 2 to Group 3.

10.3.2.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints included quit rates at 3 and 6 months following treatment termination, corrected exhaled CO levels and change from baseline, Minnesota Nicotine Withdrawal Scale results, cigarette evaluation scale questionnaire results, sensory questionnaire results, and Fagerström Test of Nicotine Dependence results. Carbon Monoxide results are summarized below with safety data, in Section 10.4.1.2.5; other secondary efficacy variables are summarized below within this section.

10.3.2.2.1. Abstinence Rates at 3 and 6 Months Following Treatment Termination

Abstinence at 3 or 6 months was defined as no cigarettes smoked from Week 7 to Month 3 (or Month 6) and corrected exhaled CO < 10 ppm from Visit 6 to Visit 11 (or Visit 12).

Abstinence rates were analyzed using several methods of imputation. A summary of results is provided in Table 10-3, below.

Table 10-3 3-Month and 6-Month Abstinence Proportions by Method of Imputation in Study Vector-201

Analysis/Imputation Method	Group 1 Proportion	Group 2 Proportion	Group 3 Proportion
3-Month Abstinence Rates			
Treatment Failure (ITT Population)[b]	20/116 (17.2%)	12/116 (10.3%)	17/114 (14.9%)
Treatment Failure Due to Lack of Efficacy (Excluding Subjects Whose Abstinence Status Could Not be Determined)	20/89 (22.5%)	12/81 (14.8%)	17/92 (18.5%)
Treatment Failure (Per Protocol Population)	17/65 (26/2%)	8/63 (12.7%)	16/76 (21.1%)
6-Month Abstinence Rates			
Treatment Failure (ITT Population)[b]	13/116 (11.2%)	9/116 (7.8%)	11/114 (9.6%)
Treatment Failure Due to Lack of Efficacy (Excluding Subjects Whose Abstinence Status Could Not be Determined)	13/88 (14.8%)	9/80 (11.3%)	11/90 (12.2%)
Treatment Failure (Per Protocol Population)	10/60 (16.7%)	5/62 (8.1%)	10/72 (13.9%)

Source: CSR for Study Vector-201, Appendix A, Tables S8, S13, and S16.

No significant differences in 3-month or 6-month abstinence rates were observed when comparing either Group 1 or Group 2 to Group 3 for any of the imputation methods.

10.3.2.2.2. Minnesota Nicotine Withdrawal Scale Results

In study Vector-201, subjects completed the Minnesota Nicotine Withdrawal Scale at each visit. Subjects were asked to rate the test "for the period of today":

Each question was analyzed individually for group differences (Group 1 or Group 2 compared to Group 3) by the two-sided Jonckheere-Terpstra test, and the change from baseline was analyzed using the Wilcoxon rank-sum test. Statistically significant results are summarized briefly by item below:

1. Depressed mood/sad:
   Group differences: Subjects in Group 1 selected slight or mild depressed mood/sad

more frequently than subjects in Group 3 at Visit 5 (p = 0.0223).
Change from Baseline: None

2. Insomnia/sleep problems, awakening at night:
   Group differences: A higher percentage of subjects in Group 1 indicated sleep problems compared to Group 3 at Visit 5 (p = 0.0002).
   Change from Baseline: At Visit 5, the mean change in Group 1 was 0.5 ± 1.4, while the mean change in Group 3 was -0.1 ± 1.1 (p = 0.0004).

3. Angry/irritable/frustrated:
   Group differences: At Visit 5, a higher proportion of subjects in Group 1 reported being angry/irritable/frustrated than in Group 3 (p = 0.004).
   Change from Baseline: At Visit 4, the mean change in Group 2 was 0.5 ± 1.2, while the mean change in Group 3 was 0.1 ± 1.2 (p = 0.0214).

4. Anxious/nervous:
   Group differences: At Visit 5, a higher proportion of subjects in Group 1 reported being anxious/nervous than in Group 3 (p = 0.005).
   Change from Baseline: None

5. Difficulty concentrating:
   Group differences: At Visits 4, 5, and 6, more subjects in Group 1 reported difficulty concentrating than subjects in Group 3 (p = 0.0189, p = 0.0044, p = 0.0372, respectively at Visits 4, 5, and 6).
   Change from Baseline: None

6. Restless/impatient:
   Group differences: None
   Change from Baseline: None

7. Increased appetite/hungry/weight problem:
   Group differences: At Visit 5, more subjects in Group 1 indicated increased appetite/hungry/weight problems than in Group 3 (p = 0.0332).
   Change from Baseline: At Visit 5, the mean change in Group 1 was 0.6 ± 1.1, while the mean change in Group 3 was 0.3 ± 1.2 (p = 0.0472).
   At Visit 9, the mean change in Group 2 was 0.4 ± 1.4, while the mean change in Group 3 was 1.0 ±1.4 (p = 0.0328).
   At Visit 10, the mean change in Group 2 was 0.3 ± 1.5, while the mean change in Group 3 was 1.0 ± 1.2 (p = 0.0176).

8. Desire or craving to smoke
   Group differences: At Visit 3, more subjects in Groups 1 and 2 experienced a desire or craving to smoke or a more intense desire or craving to smoke than subjects in Group 3 (Group 1 vs Group 3 p = 0.0428; Group 2 vs Group 3 p = 0.0105).
   At Visit 12, subjects in Group 1 experienced a desire or craving to smoke or a more intense desire or craving to smoke than subjects in Group 3 (p= 0.0466).
   Change from Baseline: At Visit 12, the mean change from Baseline in Group 1 was

-1.3 ±1.3, while the mean change from Baseline in Group 3 was -0.5 ± 1.5 ($p = 0.0279$).

10.3.2.2.3. Cigarette Evaluation Scale Questionnaire Results

Subjects completed the Cigarette Evaluation Scale at Visits 1 to 5. A total of 11 questions were asked, which were categorized into 5 component scales (satisfaction, psychological reward, nausea/dizziness, enjoy sensation, and reduced craving), and subjects were asked to rate the last cigarette smoked. Replies were tabulated and analyzed using the Jonckheere-Terpstra test and changes from baseline were calculated and analyzed using the Wilcoxon rank-sum test.

In summary, satisfaction scores at Visit 5 (after Groups 1 and 2 smoked Quest 3 for 2 weeks) were lower in Groups 1 and 2 than in Group 3. Group 1 satisfaction scores were also lower than Group 3 at Visit 4 (after smoking Quest 2 for 2 weeks). There were no significant differences in psychological reward at any visit date. Results for scores of nausea/dizziness indicated that Groups 1 and 2 experienced less nausea/dizziness compared to Group 3 at Visit 3 (after smoking Quest 1) and Group 2 also experience less nausea than Group 3 at Visit 4 (after smoking Quest 2). Not surprisingly, both Groups 1 and 2 experienced less enjoyment of the cigarette sensation than Group 3 at Visit 5 (after smoking Quest 3). Finally, Groups 1 and 2 reported greater reductions in craving than Group 3 at Visit 2 and lesser reductions in craving than Group 3 at Visit 5. In terms of change from baseline scores, Group 1 and Group 2 reported lower reductions in craving compared to Group 3 at Visits 3, 4, and 5.

10.3.2.2.4. Sensory Questionnaire Results

Subjects completed the Sensory Questionnaire at Visits 1 through 5. A total of 9 questions were asked regarding the sensations subjects experienced from puffs of the last study cigarette smoked. Replies were tabulated and analyzed using the Jonckheere-Terpstra test and change from baseline differences were calculated and analyzed using the Wilcoxon rank-sum test.

In summary, results showed that subjects liked smoking Quest 3 cigarettes considerably less and indicated that the puffs were significantly less satisfying than subjects smoking conventional cigarettes. Subjects rated all levels of Quest cigarettes as having significantly lower nicotine content than subjects smoking conventional cigarettes. Subject rated the puffs of Quest 2 and Quest 3 as considerably more different than their usual brand compared to subjects using conventional cigarettes at the same study visits.

10.3.2.2.5. Fagerström Test of Nicotine Dependence Results

The Fagerström Test for Nicotine Dependence was completed at Visits 1 to 5. Subjects answered 6 questions to calculate their level of nicotine dependence. Replies were tabulated

and analyzed using the Jonckheere-Terpstra test and change from baseline differences were calculated and analyzed using the Wilcoxon rank-sum test. No significant differences involving the individual questions were observed when comparing Group 1 with Group 3 or Group 2 with Group 3.

10.3.3. Efficacy Results from Other Published Clinical Trials

10.3.3.1. Efficacy Results from Smoking Cessation Trials

Most recently, Hatsukami *et al*[55] published a study comparing the effects of smoking Quest 3 (nicotine yield ≤0.05 mg) cigarettes or Quest 2 (nicotine yield 0.30 mg) cigarettes to the use of 4 mg nicotine lozenges for 6 weeks. Efficacy outcome measures included number of cigarettes smoked, results of the Minnesota Nicotine Withdrawal Scale, results of the Fagerström Test for Nicotine Dependence, and perceived health risk.

As shown in Figure 10-5, among subjects in the Quest 2 group, the number of cigarettes smoked per day increased significantly ($p=0.0127$ to $p<0.0001$) at each of the first 5 weeks of treatment compared to the number of usual brand cigarettes they were smoking at baseline. This is in contrast to the significantly decreased ($p=0.0043$ to $p<0.0001$) number of cigarettes smoked per day (relative to baseline) observed after Week 2 in those assigned the VLN Quest 3 (nicotine yield ≤0.05 mg) cigarettes. At Week 6, the mean number of Quest 2 cigarettes smoked per day was significantly greater than that of Quest 3 cigarettes smoked ($t = 4.73$, $p<0.0001$).

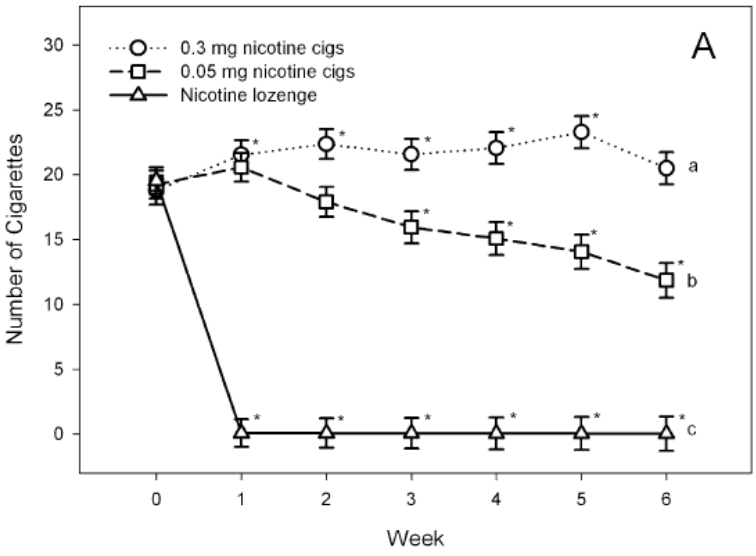


Figure 10-5 Number of Cigarettes Smoked During Treatment Period Published by Hatsukami *et al*, 2010

Four-week continuous abstinence rates and point abstinence rates for subjects who used

nicotine lozenges but no cigarettes and for subjects who used no lozenges or cigarettes are presented in Table 10-4. In this analysis, dropouts were considered treatment failures. For abstinence from cigarettes, 4-week continuous abstinence rates were highest in those receiving VLN Quest 3 cigarettes and lowest in those receiving Quest 2 cigarettes with an intermediate nicotine content, with the difference across the three groups nearly significant. Point prevalence abstinence rates followed a similar pattern, with significant differences between groups observed in abstinence rates verified by both CO and urinary cotinine concentrations. CO verified point prevalence abstinence rates were statistically significant only at the week 6 posttreatment visit and nearly significant at the Week 2 and 4 post-treatment visits.

Table 10-4 Smoking Abstinence Rates Published by Hatsukami *et al*, 2010

	Treatments						
	0.3 mg nicotine cigarettes (n = 52)		*Nicotine lozenges (n = 60)*		*0.05 mg nicotine cigarettes (n = 53)*		
	# abstinent	*%*	*# abstinent*	*%*	*# abstinent*	*%*	*P-value*
Continuous abstinence							
[1]CO verified	11	21.2	21	35.0	23	43.4	0.0508
[2]CO and cotinine verified	7	13.5	11	18.3	16	30.2	0.0913
CO verified point prevalence abstinence							
Follow-up week							
1	18	34.6	25	41.7	22	41.5	0.6954
2	17	32.7	25	41.7	29	54.7	0.0719
4	12	23.1	22	36.7	23	43.4	0.0829
6	12	23.1[a]	22	36.7[a,b]	25	47.2[b]	0.0357
CO and cotinine verified point prevalence abstinence							
Follow-up week							
2	12	23.1[a]	16	26.7[a]	24	45.3[b]	0.0298
4	8	15.4[a]	13	21.7[a]	21	39.6[b]	0.0120
6	7	13.5[a]	12	20.0[a,b]	19	35.9[b]	0.0192

[1]Carbon monoxide (CO) verified abstinence represents abstinence from cigarettes but usage of nicotine lozenge is allowed. [2]CO and cotinine verified represents abstinence from all nicotine-containing products, including nicotine lozenge.

FTND score and perceived risk for addiction score during treatment in the Hatsukami study both showed significant treatment (p = 0.0124 and <0.0001, respectively), time (p-values <0.0001) and treatment x time interaction (p-values <0.0001) effects. Significant decreases in FTND and perceived risk of addiction scores were observed for the Quest 3 and nicotine lozenge groups (all p-values < 0.001) at week 6 compared to baseline. For the Quest 2 cigarette group, perceived risk of addiction decreased significantly between baseline and week 6 (p < 0.0001); however, FTND score did not (p = 0.4810). At Week 6, significant differences between groups were found in FTND (p-values 0.0315–<0.0001) and perceived risk of addiction (p-values 0.0456–<0.0001), with the highest levels observed in those assigned to Quest 2 cigarettes and the lowest in those assigned to nicotine lozenge.

Upon cessation of usual brand cigarettes and switching to the study products, there was a significant increase in withdrawal symptoms (p-values 0.0188 to <0.0001) and no significant change in craving in all three treatment groups. Increase in nicotine withdrawal scores upon cessation of usual brand cigarettes (week 1 compared to baseline) was significantly smaller for the group assigned to VLN Quest 3 cigarettes compared to the group assigned nicotine lozenges (p = 0.0253) and nearly significantly smaller (p = 0.0917) than for those assigned to Quest 2 cigarettes. Upon cessation of the product (Week 7 compared to Week 6), significant increase in craving (p = 0.0079) and withdrawal symptoms (p < 0.0001) were observed for the Quest 2 cigarette group. In those discontinuing VLN Quest 3 cigarettes, craving increased significantly (p = 0.0138), but withdrawal symptoms did not (p = 0.2297). In those discontinuing nicotine lozenge, neither craving (p = 0.0814) nor withdrawal symptoms (p = 0.4856) were increased significantly. Change in withdrawal symptoms was significantly lower in those discontinuing VLN Quest 3 cigarettes (p = 0.0006) or nicotine lozenges (p =

0.0002) compared to those discontinuing Quest 2 cigarettes, with no significant differences in craving observed between groups.

Rezaishiraz *et al*[59] measured smoking cessation efficacy in 98 adult smokers in an unblinded Quest 3 (nicotine yield ≤0.05 mg) and a 21 mg nicotine patch for 2 weeks and the other half received only Quest 1 (nicotine yield 0.6 mg) alone. After the quit date, all smokers received nicotine patch therapy for up to 8 weeks (4 weeks of 21-mg patches, 2 weeks of 14-mg patches, and 2 weeks of 7-mg patches). All subjects also participated in three 1-hour group cessation classes, plus individual counseling on subsequent clinic visits (each individual counseling session lasting about 20 minutes). Outcome measures included self-reported smoking abstinence rates at 3 and 6 months, self-reports of cigarette cravings, and nicotine withdrawal symptoms.

Self-reported smoking abstinence rates at 3 and 6 months are shown in Figure 10-6. The abstinence rate was higher in Group 1 at both 3 and 6 months (43% vs. 34% and 28% vs. 21%, respectively), although the differences between groups were not statistically significant. Subjects who reported quitting at 3 and 6 months had statistically lower average craving scores during the 2-week post cessation period (p=0.05 at both time points).

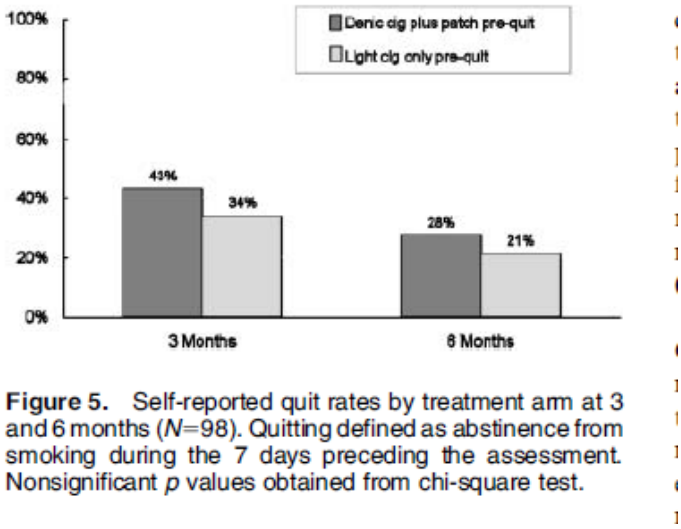
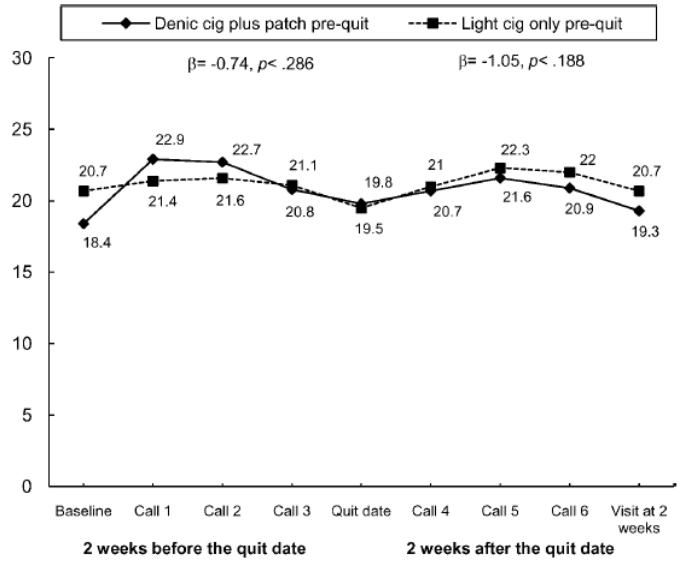


Figure 5. Self-reported quit rates by treatment arm at 3 and 6 months (*N*=98). Quitting defined as abstinence from smoking during the 7 days preceding the assessment. Nonsignificant *p* values obtained from chi-square test.

Figure 10-6 Smoking Abstinence Rates Published by Rezaishiraz *et al*, 2007.

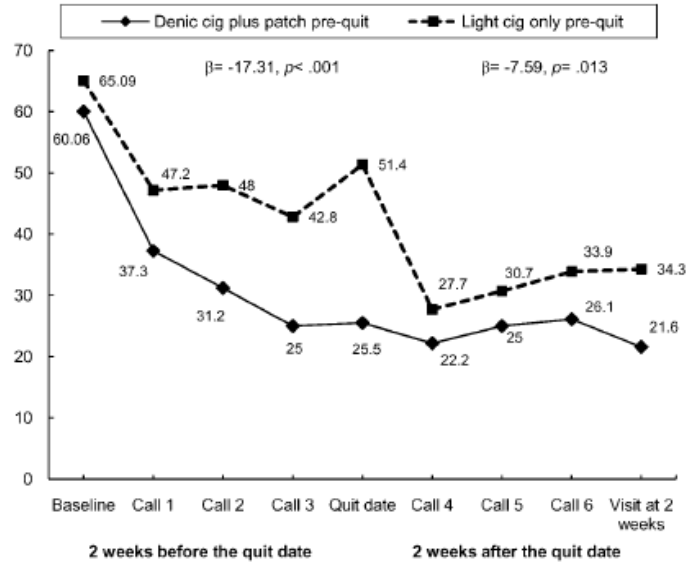
A comparison of the 2 study groups with regard to withdrawal symptoms is shown in Figure 10-7. The groups were similar in regard to the severity of withdrawal symptoms during the 2 weeks before and after the quit date.



Score (along vertical axis) = sum of scores for anger + nervousness+distractibility+impatience+appetite+insomnia+depression+desire to smoke. Beta and p values obtained from mixed-model analysis (adjusted for baseline values).

Figure 10-7 Withdrawal Symptoms Published by Rezaishiraz *et al*, 2007

Figure 10-8 shows the difference between the 2 study groups in regard to the combined craving score. Group 1, which used VLN cigarettes and nicotine patch before quitting, had lower combined craving score during the 2 weeks before ($p = 0.001$) and after ($p = 0.013$) the quit date.



Score (along vertical axis) = severity of craving x frequency of craving. Beta and p values obtained from mixed model analysis

Figure 10-8 Craving Scores Published by Rezaishiraz *et al*, 2007

Rose *et al*[60] conducted a small, unpublished study comparing Quest 3 with NRT, Quest alone, and Quest 1, Quest 2, and Quest 3 in a step-down protocol. During the first week of the study, subjects smoked only their usual brand of cigarette. From Weeks 2 through 5,

subjects gradually reduced the amount of usual brand and increased the amount of Quest 3 smoked (Groups 1 and 2), or smoked cigarettes with decreasing amounts of nicotine per cigarette (Group 3). During Weeks 6 through 11, subjects were to smoke Quest 3 exclusively. During Week 12 through Week 14, subjects were gradually weaned off Quest 3, and subjects using NRT were weaned off of nicotine replacement. In Group 2, 33% of subjects achieved 4-week continuous abstinence. Group 3 attained a 13% abstinence rate. In Group 1 no subjects achieved abstinence. No further data are available from this study.

10.3.3.2. Efficacy Results from Human Studies Conducted in a Laboratory

Several studies examined the effect of Quest 3 cigarettes on withdrawal symptoms and craving in a laboratory setting.

As described in Section 10.2.2.1, Barrett[61] examined the effects of nicotine in the absence of tobacco (through nicotine inhalers and placebo inhalers), tobacco with very low nicotine (Quest 3 cigarettes, nicotine yield ≤0.05 mg), and nicotine-containing tobacco (Quest 1 cigarettes, nicotine yield 0.60 mg) on withdrawal symptoms and other subjective measures. Briefly, 22 smokers self-administered inhaled nicotine, inhaled placebo, Quest 1 cigarettes, or VLN Quest 3 cigarettes over 4 laboratory sessions. After smoking or inhaling nicotine or placebo, subjects completed The Questionnaire of Smoking Urges-Brief version; a 10-item, self-report measure that assesses cravings using two factors: Factor 1 – intention to smoke, and Factor 2 – withdrawal/negative affect relief. Results for men and women combined for both factors and results for men vs. women for Factor 2 only are provided in Figure 10-9, below. Results show that both Quest 1 and VLN Quest 3 cigarettes were effective at reducing intention to smoke and withdrawal/negative affect and that the effect of VLN tobacco was more pronounced in women than in men.

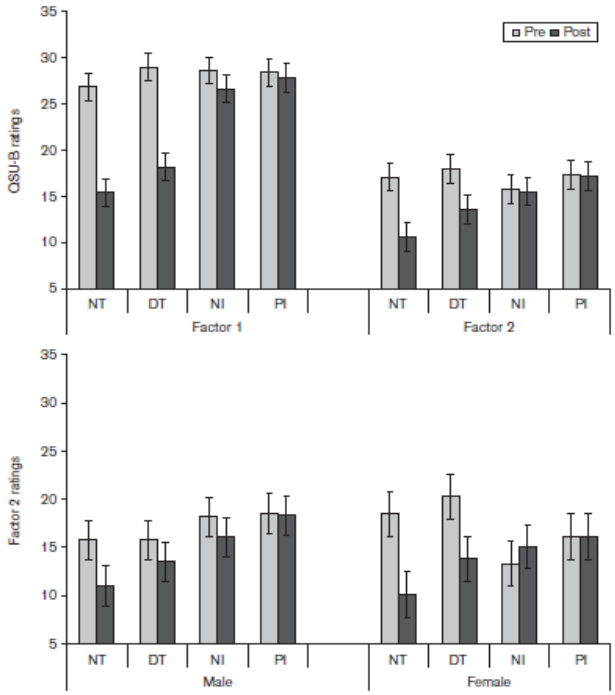
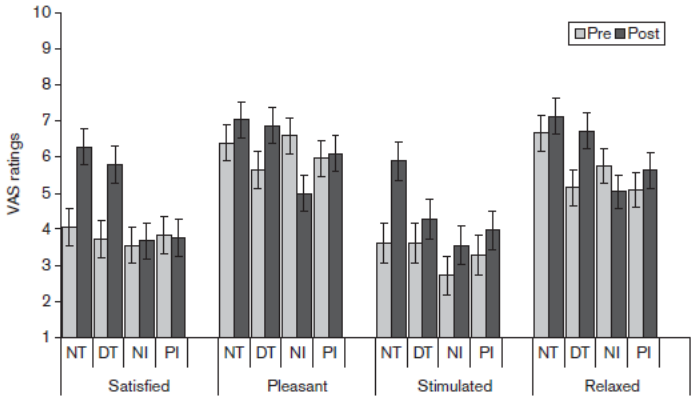


Figure 10-9
Effect of Nicotine on Withdrawal Symptoms Published by Barrett 2010

NT=nicotine tobacco, DT-denicotinized tobacco, NI-inhaled nicotine, PI-inhaled placebo, Factor 1= intention to smoke, Factor 2= withdrawal/negative affect relief

Barrett also evaluated the effects of nicotine on mood. Subjects rated themselves on scales for "satisfied", "pleasant", "stimulated" and "relaxed" after each experimental condition. Results are shown in Figure 10-10, below.



Mean (±SE) ratings of 'satisfied','pleasant','stimulated' and 'relaxed' before and after each experimental challenge. Nicotine-containing tobacco (NT) and denicotinized tobacco (DT) were rated as more satisfying than each of the inhalers; nicotine inhalers (NI) as being less pleasant than each of the other products, NT as producing more stimulation than each of the other products, and ratings of 'relaxed' were significantly greater after NT and DT administration relative to NI administration. PI, placebo inhalers; VAS, Visual Analog Scale.

Figure 10-10 Effect of Nicotine on Mood Published by Barrett 2010

As shown in this figure, smoking either VLN Quest 3 or Quest 1 was more satisfying and more relaxing than inhaling nicotine or placebo. In the figure, smoking VLN Quest 3 appears to have produced similar effects of satisfaction, pleasantness and relaxation, but not stimulation compared to Quest 1, although this direct comparison was not presented in the Barrett 2010 publication.

As described in Section 10.2.2.2, Perkins *et al*[62] compared the effects of Quest 1 (nicotine yield 0.6 mg), Quest 3 (nicotine yield ≤0.05 mg), or no cigarettes on negative affect relief after imposing a variety of stress-induced situations on 104 adult smokers. Results of effects on mood and affect are shown graphically in Figure 10-11, below. Quest 1 or VLN Quest 3 cigarettes enhanced both measures of positive affect during abstinence, but had minimal effects during other mood-induction procedures. Analysis of variance results for a questionnaire on smoking urges and the Minnesota Nicotine Withdrawal Scale were similar to those for affect. Relief of craving by smoking was generally similar across mood induction procedures, and the nicotine content of the cigarettes had little influence on these responses to smoking.

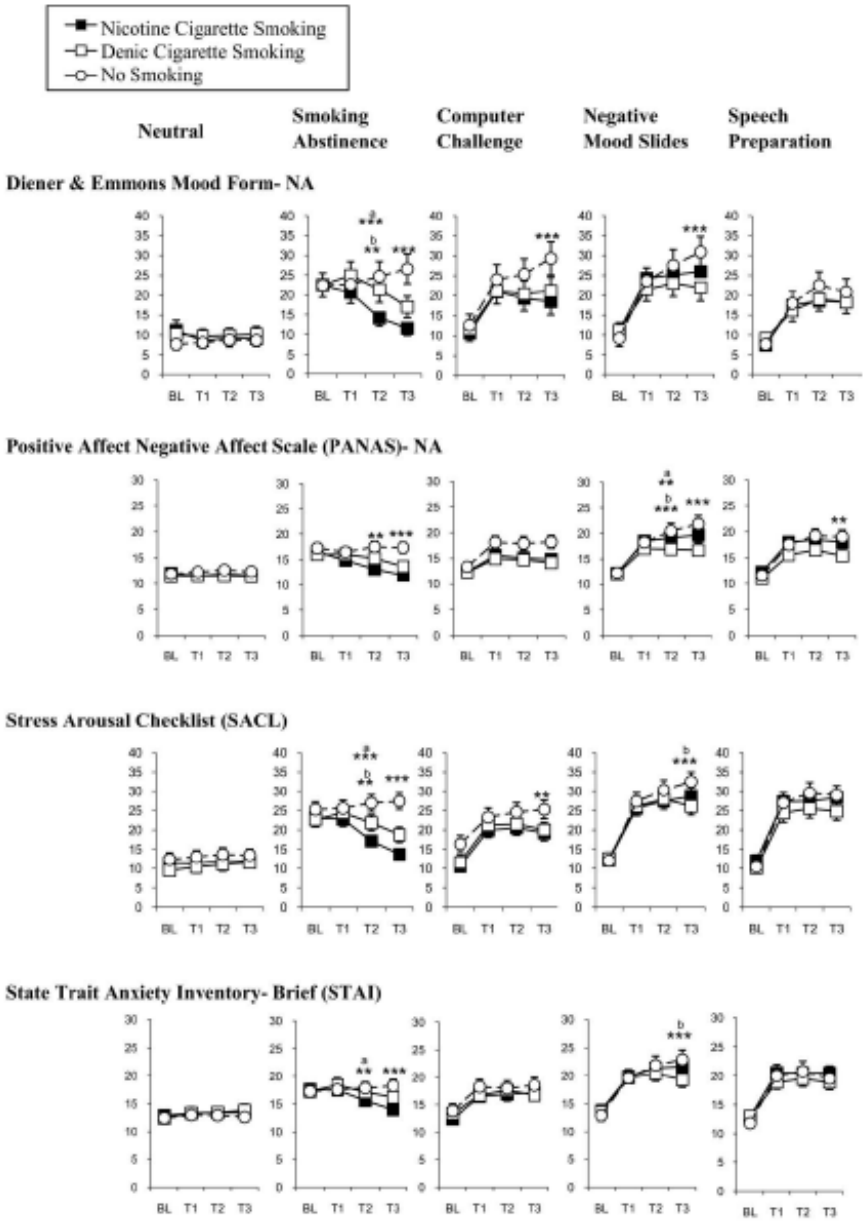


Figure 10-11 Effect of Smoking on Mood Published by Perkins *et al* 2010

10.3.4. Evaluations of Compensatory Smoking Behavior

One concern with using a nicotine reduction strategy for smoking cessation has been that smokers would compensate for reduced nicotine levels by smoking more cigarettes or smoking cigarettes more intensely. In addition to the work of Hatsukami *et al*[55] summarized in Section 10.3.3.1 above, which found no evidence of compensatory smoking when subjects smoked Quest 3 cigarettes, several other researchers have addressed the question of compensatory smoking when switching to Quest 3 or other VLN nicotine cigarettes.

Donny *et al*[66] (see also Section 10.2.2.6) evaluated the risk of compensatory smoking in an 11-day, controlled laboratory study. Thirty adult regular smokers were assigned randomly to either Quest 1 cigarettes (nicotine yield 0.6 mg), VLN Quest 3 cigarettes (nicotine yield ≤0.05 mg), or no smoking. The number of cigarettes smoked per day is shown in Figure 10-12. Subjects in the VLN Quest 3 group ("Denic") smoked fewer cigarettes, (a decrease of 3.8 cigarettes/day compared to baseline vs. an increase of 2.1 cigarettes/day in the Quest 1 cigarette group ["Nic"]), took fewer puffs per cigarette (8.8 vs. 11.5, p = 0.10), tended to leave larger butts (0.54 grams vs. 0.45 grams, p = 0.11), and had significantly lower expired CO levels. Subjects smoking VLN Quest 3 cigarettes also demonstrated a 31% decline in the number of puffs earned on a progressive ratio, a measure of the motivation to smoke, during the study. The study saw no evidence of compensatory smoking in the VLN Quest 3 cigarette group.

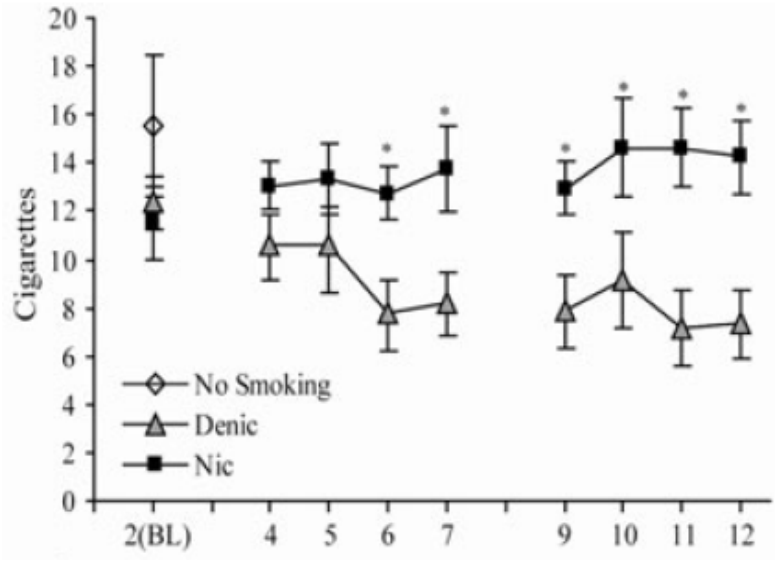


Figure 10-12 Mean (± SEM) Total Number of Cigarettes Smoked during Each Unrestricted Smoking Day Published by Donny *et al* 2007

Finally, Strasser *et al*[64,65] explored the concept of compensatory smoking in two publications (see also Sections 10.2.2.4 and 10.2.2.5). Fifty subjects participated in a single laboratory smoking session during which one cigarette of the subject's own brand, one Quest 1 cigarette, one Quest 2 cigarette, and one Quest 3 cigarette were smoked using a smoking topography machine in random order at 30-minute intervals. Selected results, not including Own brand group data are shown in Figure 10-13. Mean total puff volumes were 832.0 ml, 540.3 ml, 518.1 ml, and 570.5 ml in the Own brand, Quest 1, Quest 2, and Quest 3 cigarette groups, respectively. Mean number of puffs per cigarette were 14.3, 9.8, 9.9, and 10.0 in the Own brand, Quest 1, Quest 2, and Quest 3 cigarette groups, respectively.

In summary, although the authors concluded that this study provides evidence for the potential of compensatory smoking with low nicotine cigarettes, all Quest brand cigarette

groups were associated with lower mean total puff volume and fewer mean number of puffs per cigarette than subject's Own brand. Within the Quest groups, lower nicotine content was associated with higher total puff volume and mean number of puffs per cigarette.

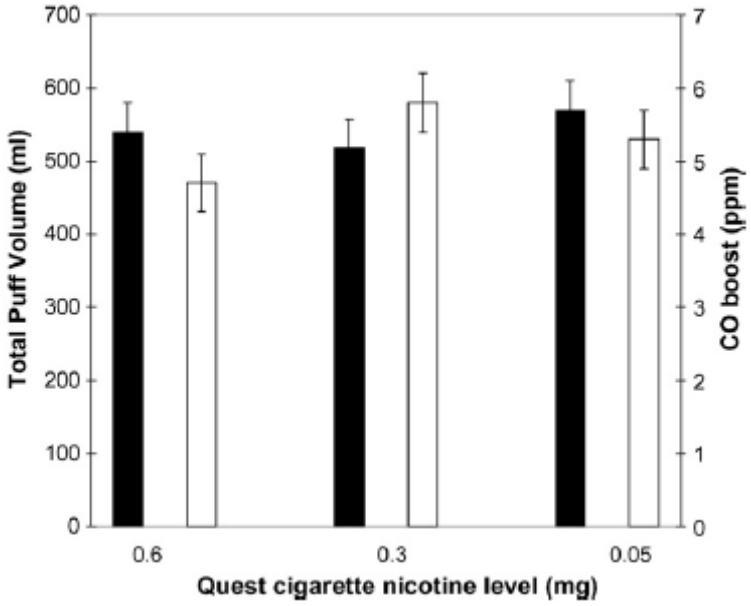


Figure 10-13 Smoking Topography Results for Quest Cigarette Groups Published by Strasser *et al* 2007

In a separate publication, Strasser also measured tar staining of cigarette filters using digital imaging to estimate total puff volume after cigarette smoking. Again, 50 subjects participated in a single laboratory smoking cession total puff volume during which one cigarette of the subject's Own brand, one Quest 1 cigarette, one Quest 2 cigarette, and one Quest 3 cigarette were smoked in random order at 30-minute intervals. Mean total puff volumes were 827.1 ml, 540.6 ml, 518.1 ml, and 570.5 ml in the Own brand, Quest 1, Quest 2, and Quest 3 cigarette groups, respectively. Thus, results were similar to the first Strasser publication discussed above, i.e., all Quest brand cigarette groups were associated with lower mean total puff volume than subject's Own brand, and within the Quest groups, lower nicotine content was associated with higher total puff volume.

10.3.5. Gender Differences in Smoking Behaviors and Response to Nicotine

There is evidence that smoking behavior is influenced by the gender of the subject.[84] Previous research has found that the smoking behavior of women is influenced less by nicotine and more by other factors. Although men and women do not differ in the physiological, cognitive, or psychomotor effects of nicotine, they may differ in their sensitivity to nicotine's reinforcing and rewarding effects.[85] This may be why women have more difficulty in quitting smoking in general.[84,86,87,88,89] Improving quit rates in women is an urgent public health need, as smoking consistently produces greater risk of the primary

smoking-related illnesses in women than in men, including lung cancer[90], myocardial infarction[91], and deterioration of lung function due to smoking.[92] Furthermore, smoking in women induces health risks related to fetal development.

There is evidence that the use of NRT has less influence on long-term quit rates in women versus men.[84] West *et al*[93] found that women tended to do worse than men after use of NRT gum, patch, or nasal spray, but better than men when using an NRT inhaler as measured by 15-week abstinence rates in an uncontrolled open-label trial. Therefore, some formulations of NRT may be more effective in women than in men. There is also evidence that smoking cessation counseling may be more effective in women than in men.[87]

One approach in smoking cessation therapies is to provide substitutes that simulate the sensory effects of smoking. Standard counseling recommends strategies such as sucking on a straw or consuming carrot sticks or cinnamon sticks as substitutes for the motor effects of smoking.[84] However, these methods mimic only the motor effects of smoking, but not other sensory effects such as olfactory and taste stimuli and sensations to the throat. VLN cigarettes match almost all of the effects of smoking other than nicotine intake and therefore, may be particularly useful as a smoking cessation tool in women. This hypothesis is supported by Barrett *et al*[61] (see also Sections 10.2.2.1 and 10.3.3.2), who found that women experienced greater relief of withdrawal-related cravings than men after smoking Quest cigarettes.

Because of possible gender differences in smoking cessation rates, the first clinical trial of X-22 Smoking Cessation Product will balance the gender of subjects across treatment groups. An exploratory objective of this study will be to compare the influence of gender on smoking cessation rates.

10.3.6. Efficacy Conclusions from Clinical Trials

Previous studies of VLN cigarettes for smoking cessation indicate that a product that delivers the sensory and behavioral cues associated with cigarette use but does not deliver a satisfying amount of nicotine breaks the association of those cues with the contingent delivery of nicotine reinforcement and promotes extinction of smoke cues. Studies described above in Sections 10.3.2.1 and 10.3.3.1 have evaluated the use of VLN cigarettes for periods of 2 to 6 weeks and used alone or in combination with NRT. It appears that short term use for 2 weeks was less effective than longer term use over 6 weeks.[59,55] Although some studies have found significant efficacy with concomitant use of NRT, especially when the NRT was begun at least 2 weeks before the quit date, other studies have found that VLN cigarettes alone are quite effective, with abstinence rates up to 43%.[55,53] Smoking VLN cigarettes for at least 6 weeks alone also seems to be more effective that using a step-down approach (e.g., 2 weeks of 0.6 mg nicotine cigarettes, followed by 2 weeks of 0.3 mg nicotine cigarettes, followed by 2 weeks of ≤0.05 mg nicotine cigarettes).[53,54,55]

Although there has been concern that when smokers switch from their usual brand to reduced nicotine cigarettes, compensatory increases in smoking may occur, when switching to Quest 3 nicotine yield ≤0.05 mg cigarettes compensatory smoking has not been substantiated. While short term studies have been inconclusive[64,65,66], several long-term studies have found that smokers who switch to VLN cigarettes voluntarily decrease the number of cigarettes they smoke over time.[55,23]

Finally, numerous studies have found that acute withdrawal effects and cravings after short term smoking abstinence, as evaluated in a laboratory setting, are decreased as effectively or nearly as effectively with VLN cigarettes as with regular cigarettes.[61,62,81,17,16,21,14] Furthermore, smoking VLN cigarettes was more effective than inhaling nicotine at relieving cravings and other withdrawal symptoms.[61] During a longer term study of smoking cessation, smoking VLN cigarettes were also associated with less withdrawal effects as compared to nicotine lozenges.[55]

10.4. Safety

10.4.1. Clinical Safety of X-22 Smoking Cessation Product

10.4.1.1. Overview

Like conventional cigarettes, X-22 cigarettes are toxic and pose health risks to subjects, namely cancer, cardiovascular disease, respiratory disease and reproductive effects3. Furthermore, it has been suggested that nicotine may mitigate some of the negative effects of other cigarette smoke components[83,51].

During clinical studies, X-22 cigarettes will be provided in a limited amount only to confirmed smokers. Previous clinical studies (see Section 10.2) have found that, when smoking VLN cigarettes, smokers generally consume fewer cigarettes per day than when they smoke their usual brand. It is therefore expected that the exposure of subjects to smoke components in the proposed IND trial will be no greater than if they did not participate and continued to smoke their usual brand.

Because the purpose of X-22 Smoking Cessation Product is for smoking cessation, and not a substitute for other cigarettes, if approved its use will be short term and monitored by a physician as a prescription product. Therefore, it is anticipated that the long term benefits of smoking cessation that are provided by X-22 should out-weight its short term health risks to subjects.

Although numerous studies of cigarettes containing VLN tobacco have been published (see Section 10.2), most of these publications focused on efficacy or subjective effects and did not address safety. Studies that provided safety information are discussed in the following sections.

10.4.1.2. Safety Results from Vector-201 Phase II CSR

10.4.1.2.1. Summary

The safety of cigarettes manufactured with VLN tobacco was systematically evaluated in one IND clinical trial: Vector-201, A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of Quest Alone and in Combination with Nicotine Replacement Therapy as a Smoking Cessation Aid.[53,54]

As summarized in Sections 10.2.1.1 and 10.3.2, a total of 346 healthy smokers who were motivated to quit smoking were enrolled in the trial and followed over 8 months. Subjects were randomized to one of the following groups:

- Group 1: Quest 1, Quest 2, and Quest 3 plus NRT patch
- Group 2: Quest 1, Quest 2, and Quest 3 plus placebo patch
- Group 3: Conventional active control cigarettes plus NRT patch

A total of 85 subjects completed the study. The median time to drop out was 12.8 weeks for Group 1, 10.5 groups for Group 2, and 14.2 weeks for Group 3. The most common reasons for drop out were failure to quit smoking (38.4%), lost to follow-up (17.1%), and subject's request/withdrawal of consent (13.3%).

Safety was evaluated by monitoring concomitant medications, adverse events, vital signs, and physical examinations. Exhaled carbon dioxide levels were also measured.

10.4.1.2.2. Adverse Events

A summary of AEs reported during study Vector-201 is presented below in Table 10-5. The number of subjects reported treatment emergent AEs was lowest in Group 2 (Quest cigarettes plus placebo patch). There were also fewer SAEs and AEs leading to discontinuation of the study in Group 2 compared to Group 1 or Group 3. A complete list of all AEs reported during the study is provided in Table 10-5.

Table 10-5 Summary of Adverse Events in Clinical Study Vector-201

Adverse Event Category	Group 1 (N=116)	Group 2 (N=116)	Group 3 (N=114)
Treatment-emergent AEs	71 (61.2%)	61 (52.6%)	69 (60.5%)
Treatment-related AEs	51 (44.0%)	32 (27.6%)	42 (36.8%)
SAEs	2 (1.7%)	0	1 (0.9%)
Treatment-related SAEs	0	0	0
AEs leading to study product stopped	4 (3.4%)	1 (0.9%)	4 (3.5%)
AEs leading to study discontinuation	3 (2.6%)	2 (1.7%)	3 (2.6%)

N=number of subjects who received treatment; %=n/N x 100; AE=adverse event; SAE=serious adverse event
Source: CSR for Study Vector-201, in-text Table 25, Summary of Adverse Events (Safety Population)

A summary of all AEs reported in 2 or more subjects in any treatment group by system organ class is provided in Table 10-6. The types and frequencies of most AEs were similar across the 3 treatment groups. However, administration site conditions were higher in the 2 groups receiving the nicotine patch and lowest in Group 2, which received Quest cigarettes plus the placebo patch. Infections and infestations occurred in more subject receiving regular cigarettes plus nicotine patch (Group 3), although 4 subjects in Group 2 compared with 0 subjects in Groups 1 and 2 experienced bronchitis. More subjects in Group 2 also experienced headache (7, 12, and 4 subjects in Groups 1, 2, and 3, respectively). However, rates of insomnia were higher in the groups receiving the nicotine patch (15, 6, and 14 in Groups 1, 2, and 3, respectively), and more subjects in Group 1 experienced anxiety and irritability (anxiety: 8, 2, and 3 in Groups 1, 2, and 3, respectively, and irritability: 7, 2, and 2, in Groups 1, 2, and 3, respectively).

Table 10-6 Adverse Events Reported in Study Vector -201 in Two or More Subjects in Any Treatment Group

System Organ Class Preferred Term	Group 1 (N=116) n (%)	Group 2 (N=116) n (%)	Group 3 (N=114) n (%)
Subjects with any adverse events	71 (61%)	61 (53%)	69 (61%)
Gastrointestinal Disorders			
Nausea	8 (7%)	7 (6%)	5 (4%)
Diarrhea	2 (2%)	3 (3%)	2 (2%)
Dyspepsia	3 (3%)	1 (<1%)	2 (2%)
Stomach Discomfort	1 (<1%)	0	2 (2%)
Abdominal Pain	2 (2%)	1 (<1%)	1 (<1%)
Vomiting	2 (2%)	3 (3%)	0
General Disorders and Administration Site Conditions			
Application Site Pruritus	8 (7%)	1 (<1%)	5 (4%)

System Organ Class **Preferred Term**	**Group 1 (N=116) n (%)**	**Group 2 (N=116) n (%)**	**Group 3 (N=114) n (%)**
Fatigue	3 (3%)	3 (3%)	3 (3%)
Application Site Dermatitis	1 (<1%)	1 (<1%)	2 (2%)
Application Site Discomfort	0	0	2 (2%)
Chest Discomfort	1 (<1%)	0	2 (2%)
Oedema Peripheral	2 (2%)	1 (<1%)	1 (<1%)
Application Site Discoloration	2 (2%)	0	0
Application Site Erythema	4 (3%)	2 (2%)	0
Immune System Disorders			
Hypersensitivity	0	0	2 (2%)
Seasonal Allergy	0	0	2 (2%)
Infections and Infestations			
Nasopharyngitis	3 (3%)	2 (2%)	8 (7%)
Upper Respiratory Tract Infection	2 (2%)	2 (2%)	6 (5%)
Tooth Abscess	0	0	2 (2%)
Ear Infection	0	2 (2%)	1 (<1%)
Bronchitis	0	4 (3%)	0
Sinusitis	2 (2%)	1 (<1%)	0
Injury, Poisoning and Procedural Complications			
Post Procedural Pain	2 (2%)	1 (<1%)	1 (<1%)
Arthropod Bite	0	2 (2%)	0
Contusion	0	2 (2%)	0
Investigations			
Blood Cholesterol Increased	2 (2%)	0	0
Metabolism and Nutrition Disorders			
Increased Appetite	0	0	3 (3%)
Musculoskeletal and Connective Tissue Disorders			
Chest Wall Pain	0	1 (<1%)	2 (2%)
Muscle Spasms	0	1 (<1%)	2 (2%)
Pain in Extremity	1 (<1%)	0	2 (2%)
Shoulder Pain	0	1 (<1%)	2 (2%)
Myalgia	1 (<1%)	2 (2%)	1 (<1%)
Back Pain	4 (3%)	6 (5%)	0
Neck Pain	1 (<1%)	3 (3%)	0
Pain in Jaw	2 (2%)		
Nervous System Disorders			

System Organ Class **Preferred Term**	**Group 1 (N=116) n (%)**	**Group 2 (N=116) n (%)**	**Group 3 (N=114) n (%)**
Headache	7 (6%)	12 (10%)	4 (4%)
Dizziness	4 (3%)	2 (2%)	2 (2%)
Paraesthesia	1 (<1%)	0	2 (2%)
Disturbance in Attention	2 (2%)	1 (<1%)	1 (<1%)
Psychiatric Disorders			
Insomnia	15 (13%)	6 (5%)	14 (12%)
Abnormal Dreams	4 (3%)	1 (<1%)	4 (4%)
Anxiety	8 (7%)	2 (2%)	3 (3%)
Depression	1 (<1%)	3 (3%)	3 (3%)
Irritability	7 (6%)	2 (2%)	2 (2%)
Initial Insomnia	0	2 (2%)	0
Nervousness	4 (3%)	0	0
Nightmare	2 (2%)	1 (<1%)	0
Respiratory, Thoracic and Mediastinal Disorders			
Cough	4 (3%)	2 (2%)	7 (6%)
Pharyngolaryngeal Pain	5 (4%)	2 (2%)	3 (3%)
Dyspnea	3 (3%)	2 (2%)	2 (2%)
Sinus Congestion	0	0	2 (2%)
Productive Cough	2 (2%)	2 (2%)	1 (<1%)
Respiratory Track Congestion	0	2 (2%)	0
Skin and Subcutaneous Tissue Disorders			
Dermatitis Contact	1 (<1%)	1 (<1%)	2 (2%)
Rash	0	0	
Urticaria	2 (2%)	1 (<1%)	1 (<1%)
Vascular Disorders			
Hot Flush	0	0	2 (2%)
Hypertension	2 (2%)	0	1 (<1%)

Source: CSR for Study Vector-201, Appendix A, Table S33

A total of 11 subjects either stopped taking the study product due to an AE or were discontinued from the study due to an AE. These events are summarized in Table 10-7, below. As shown in this table, most of the AEs leading to discontinuation in Groups 1 and 3 appear to be related to use of the nicotine patch and 1 of the events in Group 2 appears to be related to the placebo patch, not the use of the study cigarettes.

Table 10-7 Summary of Adverse Events Leading to Stopping of Study Product or Study Discontinuation in Clinical Study Vector-201

Group # Subject #	Adverse Event(s)	Stopped Study Product Due to AE?	Discontinued from Study Due to AE?
Group 1 01295	Nausea	Yes	Yes
Group 1 03051	Nausea/ Vivid dreams	Yes	No
Group 1 03067	Rash, itchiness under patch site	Yes	Yes
Group 1 04015	Chest urticaria	Yes	No
Group 1 03064	Chest pressure	No	Yes
Group 2 03060	Itchiness on chest and legs/ redness under patch site	Yes	Yes
Group 2 04003	Shortness of breath/ Chest pain/ Insomnia	No	Yes
Group 3 01311	Headache and cough	Yes	Yes
Group 3 02043	Contact dermatitis	Yes	Yes
Group 3 05110	Localized muscle aches under patch	Yes	Yes
Group 3 05245	Rash under patch	Yes	No

AE=adverse event; SAE=serious adverse event

Source: CSR for Study Vector-201, in-text Table 26, Subjects Who Experienced Adverse Events Directly or Indirectly Leading to Study Discontinuation

SAEs were reported for 3 subjects: 2 subjects in Group 1 and 1 subject in Group 3. In Group 1, chest discomfort and increased blood pressure were reported for Subject 03064 and bladder carcinoma was reported for Subject 01084. In Group 3, fibula and tibia fractures were reported for Subject 05121. None of the SAEs were considered treatment related.

10.4.1.2.3. Vital Signs

Vital signs were measured at each study visit in Study Vector-201. Clinically significant changes in heart rate or blood pressure were reported as AEs. The clinical study report states that other than the reported adverse events, no other clinically significant changes in vital signs were reported. Overall, mean differences in heart rate and blood pressure between treatment groups and over time were small and without pattern.

10.4.1.2.4. Physical Examination Results

During this study, a large percentage of posttreatment physical examination results were reported as "unknown" during this study. Therefore, results are difficult to interpret.

10.4.1.2.5. Carbon Monoxide Levels

The corrected mean CO levels for each treatment group at each study visit are presented in Figure 10-14, below. The quit smoking date was at Visit 5 and subsequent visits took place at 2-week intervals. There were significant differences ($p \leq 0.05$) when comparing Group 1 to Group 3 at Visit 3 and Visit 4, and when comparing Group 2 to Group 3 at Visit 3. There were no significant differences between groups at other time points. The study schedule dictated that subjects smoke Quest 1 during weeks 1 to 2 (between Visits 2 to 3), Quest 2 during weeks 3 to 4 (between Visits 3 to 4), and Quest 3 during Weeks 5 to 6 (between Visits 4 to 5). Therefore, during the period that subjects smoked Quest 3, it appears that Groups 1 and 2 had somewhat lower CO levels than the 'regular' cigarette group (Group 3); however differences were not significant. In their report of this study, Becker *et al*[99] noted that:

> While CO levels remained constant from Week 1 to Week 3 in the active control plus NRT group (M change 520.2 ppm, standard deviation [SD] 510.25), CO levels increased nonsignificantly by 1.5 ppm (SD 511.27) with Quest 1 cigarettes in the Quest plus NRT group (F[1,220]51.46, p5.22) and by 2.5 ppm (SD510.69) in the Quest plus placebo patch group (F[1,221]53.64, p5.06). By Week 5, CO levels remained constant in the active control plus NRT group (M change vs. Week 1 of 0.2 ppm, SD510.59). There was, however, a trend for CO levels to increase upon smoking Quest 2 cigarettes, by 2.9 ppm (SD511.25) in the Quest plus NRT group (F[1,213]53.26, p5.07) and by 2.9 ppm (SD510.82) in the Quest plus placebo patch group (F[1,215]53.39, p5.07). No differential increase in CO levels was seen with Quest 3 cigarettes [see Week 7 data].

A summary of mean CO values at each study visit for each group is provided in Appendix A, Table S19 of the clinical study report for this study.

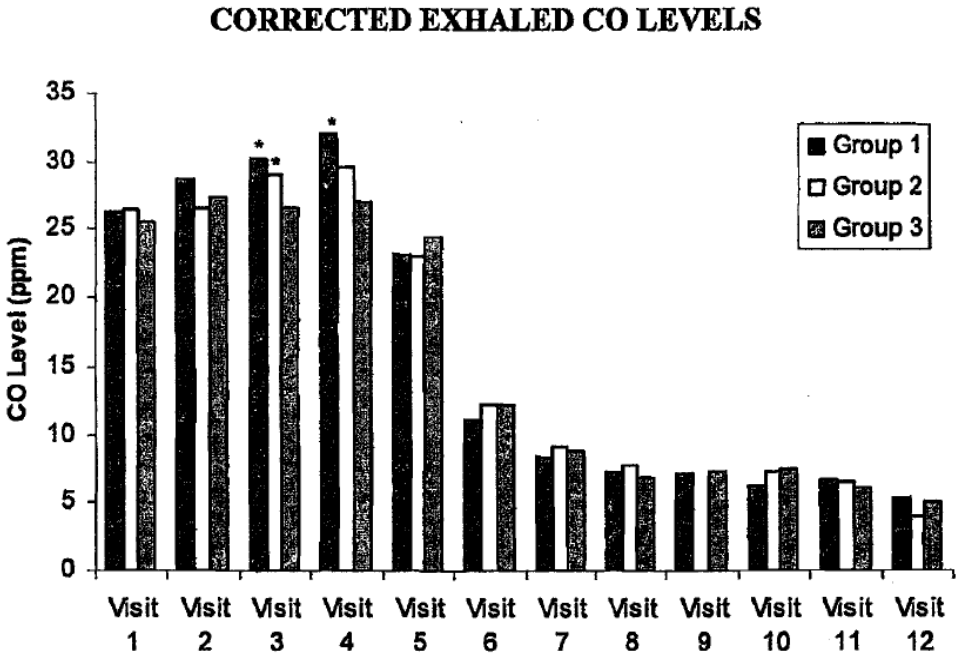


Figure 10-14 Exhaled Carbon Monoxide Data Reported in Study Vector-201

10.4.1.3. Safety Information from Other Published Clinical Studies

10.4.1.3.1. Adverse Events

Only one of the published studies of Quest cigarettes summarized in Section 10.2 provided information regarding adverse events in study subjects. Rose *et al*[80] reported that 24 hours after subjects completed 2 laboratory sessions involving smoking Quest 1 and Quest 3 cigarettes after rinsing the mouth with silver acetate or placebo solution, each participant was contacted via telephone and asked questions regarding any adverse events or signs of argyria (a bluish-tray discoloration of the skin). No occurrences were reported.

10.4.1.3.2. Vital Signs

A study by Cobb *et al*[63] (see section 10.2.2.3) evaluated the acute effects of various noncombustible PREPs (Ariva compressed tobacco tablet, Camel Snus, Marlboro Snus, and Commit® 2 mg Nicotine Lozenge) and combustible tobacco products (Subjects' OWN brand, SHAM [puffing on an unlit cigarette], and Quest 3 [nicotine yield ≤0.05 mg]) on heart rate in a laboratory setting.

This study found that mean heart rate increased in the Quest 3 group less than in a group of subjects smoking regular cigarettes and to a similar level to the group using Camel Snus, and that increase was not sustained after a second exposure to the Quest product. For the OWN

group, mean baseline heart rate was 67.8 bpm (SEM 1.9) and increased significantly to 82.3 bpm (SEM 2.3) 5 minutes after the first product administration and remained elevated at 15 and 30 minutes post administration; the same pattern was observed after a second administration of OWN cigarettes. For the Quest group, mean baseline heart rate was 68.7 bpm (SEM 2.1), which increased significantly to 73.1 bpm (SEM 2.1) 5 minutes after the first, but not the second product administration. Mean changes in heart rate were small or did not occur in subjects assigned to SHAM (puffing on an unlit cigarette), ARIVA, COMMIT lozenge, or Marlboro Snus groups.

10.4.1.3.3. Carbon Monoxide Exposure

Carbon monoxide exposure from VLN cigarettes does not appear to be any greater than that from regular cigarettes. Strasser *et al*[64] reported CO levels in smokers after smoking 1 of their own brand cigarettes, 1 Quest 1 cigarette, 1 Quest 2 cigarette, and 1 Quest 3 cigarette with 30 minutes between cigarettes (see also Section 10.2.2.5). CO levels were obtained prior to and 4 minutes after smoking each cigarette. Results for the Quest cigarettes, but not the Own brand cigarettes are presented with plasma nicotine levels in Figure 10-15, below. Mean CO boost measures were 5.3 ppm (S.D. = 2.5), 5.8 ppm (SD = 2.6), and 4.7 ppm (SD = 2.7) for the ≤0.05 mg nicotine, 0.3 mg nicotine and 0.6 mg nicotine cigarettes, respectively [$F(2,47) = 5.43$, $p = 0.01$]. These data show that smoking the VLN cigarette resulted in CO levels that were not significantly different from smoking cigarettes with higher nicotine contents.

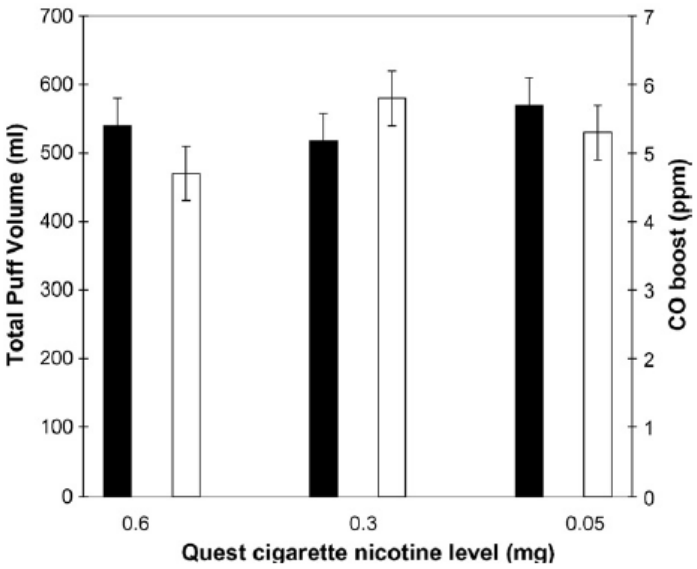


Fig. 1. The effect of cigarette nicotine level on total puff volume [(ml; solid bars; left *y*-axis); $F(2, 47) = 5.73$, $p = 0.006$, $N = 50$] and mean carbon monoxide boost [(ppm; open bars; right *y*-axis); $F(2, 47) = 5.43$, $p = 0.01$, $N = 50$]. Data presented as mean (±95% confidence interval).

Figure 10-15 Exhaled CO Levels Published by Strasser *et al*, 2007

Donny *et al*[66] compared groups who smoked Quest 1 (nicotine yield 0.6 mg) cigarettes, Quest 3 (nicotine yield ≤0.05 mg) cigarettes, or no cigarettes over a 2-week study period. They found lower CO levels in subjects who smoked Quest 3 cigarettes ("Denic") compared to Quest 2 cigarettes ("Nic") over a 2-week study period. Results are presented in Figure 10-16, below. The differences between the groups were statistically significant. Contrasts focused on the 2 smoking groups indicated that the Quest 2 cigarette group had higher CO levels than the VLN Quest 3 cigarette group overall ($p = 0.026$) and that the differences between smoking groups increased during the day ($p < 0.001$).

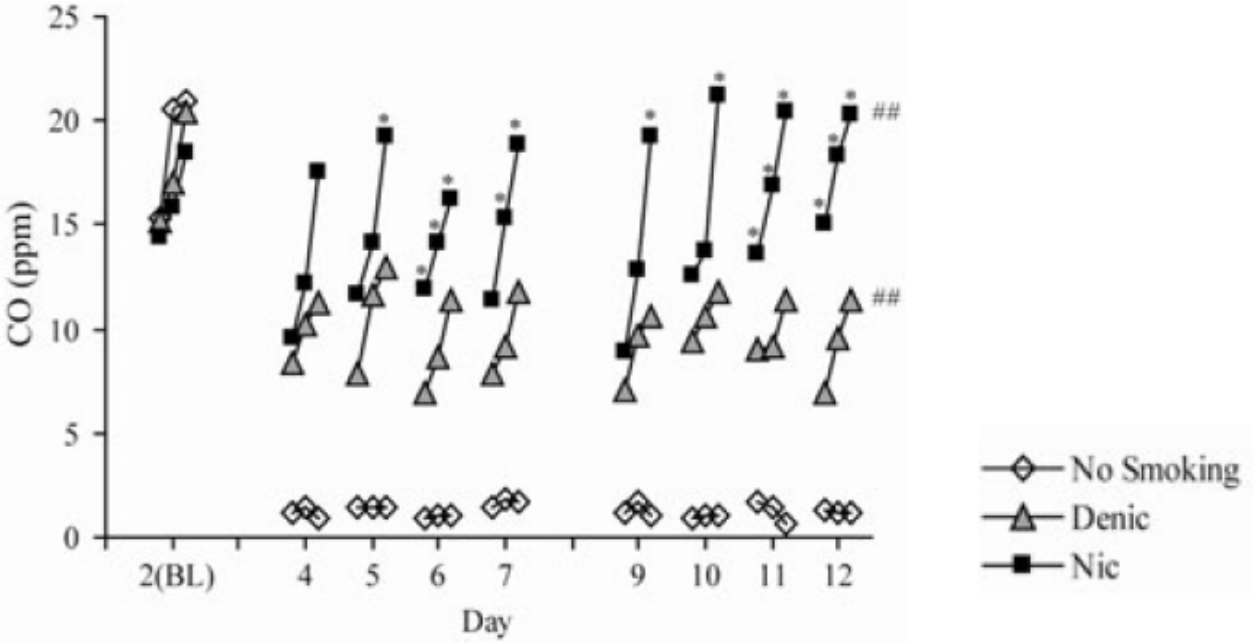


Figure 10-16 Carbon Monoxide Levels in Smokers Published in Donny *et al*, 2007

Cobb *et al*[63] (see section 10.2.2.3) evaluated the acute effects of various noncombustible PREPs (Ariva compressed tobacco tablet, Camel Snus, Marlboro Snus, and Commit® 2 mg Nicotine Lozenge) and combustible tobacco products (Subjects' OWN brand, SHAM [puffing on an unlit cigarette], and Quest 3 [nicotine yield ≤0.05 mg]) on expired CO in a laboratory setting. Each subject participated in each condition over 7 separate laboratory sessions. Mean CO values appeared to be slightly lower in the Quest 3 condition compared to the OWN brand condition. After the first product administration, the study found mean expired CO values in the OWN condition of 12.8 parts ppm (SEM 0.7) and mean expired CO values in the Quest 3 condition of 11.0 ppm (SEM 0.6) and after the second administration mean values were 17.5 ppm (SEM 1.0) in the OWN condition and 14.4 ppm (SEM 0.8) in the Quest 3 condition. There were no significant changes in any other condition.

10.4.1.3.4. Platelet Activation

Girdhar *et al*[94] conducted a study that evaluated platelet activation after cigarette smoking (see also Section 10.2.2.7). Chronic smoking is associated with increased risk of cardiovascular disease such as arterial venous thrombosis, stroke, and myocardial infarction. Platelets play an important role in both the initiation and propagation of clot formation and inflammatory response. The authors considered that baseline platelet activation state (PAS) is an important marker of cardiovascular risk and conducted this study to evaluate the effect of smoking different kinds of cigarettes on PAS.

Thirty-two smokers smoked 3 cigarettes with moderately reduced nicotine (Quest 1, nicotine yield 0.6 mg) in 1 hour. Following this first smoking hour, 16 of the subjects continued smoking with 5 of the same cigarettes over the next 1.5 hours, while the other 16 subjects smoked 5 VLN cigarettes (Quest 3, nicotine yield ≤0.05 mg). (Consuming 8 cigarettes over 2.5 hours equates to approximately 51 cigarettes/16-hour day and 5 VLN cigarettes over 1.5 hours equates to approximately 53 cigarettes/16-hour day.) Platelet surface expression of P-selectin, a marker for platelet activation, was determined by flow cytometry on samples drawn at the end of each period. The percentage of platelets scored as activated from baseline to posttreatment was 1.85% to 3.59% after smoking VLN cigarettes compared to an increase from 1.84% to 2.46% after smoking moderately reduced nicotine cigarettes. Statistical analyses showed this increase to be significantly different from baseline in both groups ($p < 0.01$) and the interaction between multiple measures of PAS with time and nicotine content of cigarettes was also significant ($p < 0.02$). The authors concluded that nicotine modulates platelet activation *in vivo* in smokers, and may significantly moderate the risk of cardiovascular disease caused by non-nicotine smoke components. Results are presented in Figure 10-17, below. The clinical significance of these results is unknown.

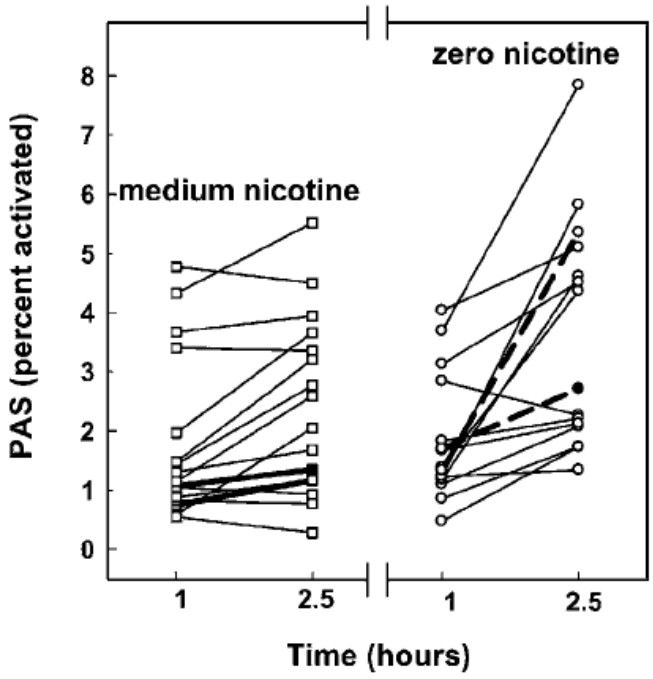


Figure 10-17 Platelet Activation Scores Published by Girdhar *et al*, 2008b

10.4.2. Clinical Safety Information about Menthol in Cigarettes

The 2011 Tobacco Products Scientific Advisory Committee's (TPSAC) "Draft Report and Recommendations on Menthol in Cigarettes"[34] found that the availability of menthol cigarettes increases initiation of smoking and reduces cessation, thereby increasing the number of people who are smoking. This increase in the number of smokers represents an adverse impact of the availability of menthol cigarettes on public health. However, they also concluded that the evidence does not indicate increased disease risks in smokers of menthol cigarettes compared to non-menthol cigarettes. This report summarizes several studies comparing the safety of menthol versus non-menthol cigarettes.

The TPSAC report[34] summarizes findings from a series of studies involving measurements of cardiovascular parameters after smoking menthol versus non-menthol cigarettes. They describe differing patterns of short-term response using echocardiography and measures of vascular response. The participants were healthy young volunteers. These findings have uncertain implications with regard to the comparative cardiovascular disease risks of smokers of menthol cigarettes versus non-menthol cigarettes. One study summarized investigated the effects of menthol in cigarettes by having volunteers smoke VLN cigarettes, with and without menthol. Using electroencephalogram and heart rate as outcome measures, they did not identify differences in response to the menthol-containing and non-menthol-containing cigarettes.

Another study summarized by the Tobacco Products Scientific Advisory Committee also examined biomarkers of potential harm, including markers of oxidative stress (i-epi-

prostaglandin-F2 alpha, 8-isoprostaglandin F2 alpha-V1), inflammation (white blood cell count, fibrinogen, C-reactive protein, monocyte chemotactic adhesion molecule-1, soluble vascular cell adhesion molecule-1), coagulation (platelets, fibrinogen, von Willebrand factor, 11-dehydrothroboxane-B2), lipids (triglycerides, LDL cholesterol, HDL cholesterol, total cholesterol, oxidized LDL, lipoprotein-associated phospholipase A2) and metabolism (glucose, adiponectin, leptin). No significant effects of menthol smoking on these biomarkers were observed.

In summary, it is not expected that the addition of menthol to X-22 cigarettes will have an adverse effect on the safety of this product.

10.4.3. Exposure to Very Low Nicotine Cigarettes

Quest 3 and Quest 3 Menthol VLN cigarettes were sold commercially in the United States from approximately December 2002 to December 2007. According to filings by Vector Group Ltd. (parent company of Vector Tobacco Inc.) with the Securities and Exchange Commission, sales of Quest by Vector Tobacco Inc. from 2002 to 2007 were approximately $60 million. This equates to approximately 26 million packs (520 million cigarettes) sold or 14,246 pack-a-day smokers using Quest for 5 years.

Since Quest was sold by Vector Tobacco in only 8 U.S. states (New York, New Jersey, Pennsylvania, Ohio, Indiana, Illinois, Michigan, and Arizona) representing 31% of U.S. smokers, approximately 1.4 million smokers tried Quest. Assuming the trial rate was even among Quest 1, Quest 2, and Quest 3 (which was labelled "nicotine free" on the packaging), approximately 467,000 smokers used Quest 3 VLN cigarettes.

Subjects' exposure to VLN cigarettes in published studies and one unpublished report is summarized in Table 10-8. Nearly 1000 subjects have received VLN cigarettes under controlled conditions. Most of these smoked VLN cigarettes in a laboratory setting during 1 to 7 sessions. Others smoked VLN cigarettes for periods of up to 13 weeks.

Table 10-8 Summary of Published Human Studies with Very Low Nicotine Tobacco

References	No. of Subjects In Study	No. of Subjects receiving Quest 3/ completed treatment	Duration of Quest 3 Cigarette Exposure	Study Topics						
				Smoking Cessation	Craving/ Withdrawal	Response to Cues	Cigarette Perception	Smoking Behavior	Exposure/ Toxicology	Other
Becker *et al*. (2008); Drgon *et al*. (2009); Vector-301 CSR	346	232/200	2 weeks (following 4 weeks of Quest 1 and Quest 2 cigarettes)	X	X					
Hatsukami *et al* (2010)	165, 1/3 smoked VLN cigarettes	49/32	6 weeks	X	X				X	
Rezaishiraz *et al*. (2007)	98, 1/2 smoked VLN cigarette	51/32	2 weeks	X	X					
Personal communication from Jed Rose, 2003	45	45/?	Up to 13 weeks	X	X		X	X	X	
Barrett (2010)	22	20/20	1 laboratory session		X			X		
Perkins *et al*. (2010)	104, 1/3 smoked VLN cigarettes	≈35/?	5 laboratory sessions		X					X
Cobb *et al*. (2010)	28	28/28	1 laboratory session		X			X	X	
Strasser *et al*. (2006); Strasser *et al*. (2007)	50	50/50	1 laboratory session				X	X		
Donny *et al*. (2007)	30, 1/3 smoked VLN cigarettes	≈10/?	11 days		X			X		
Girdhar *et al*. (2008)	32, 1/2 smoked VLN cigarettes	16/16	1 laboratory session						X	

References	No. of Subjects In Study	No. of Subjects receiving Quest 3/ completed treatment	Duration of Quest 3 Cigarette Exposure	Study Topics						
				Smoking Cessation	Craving/ Withdrawal	Response to Cues	Cigarette Perception	Smoking Behavior	Exposure/ Toxicology	Other
Perkins *et al*. (2004)	96	≈48/48	1 laboratory session				X	X		
Naqvi and Bechara (2005)	20	20/20	1 laboratory session				X			
Rukstalis *et al*. (2005)	26	≈26/26	1 laboratory sessions					X	X	
Perkins *et al*. (2006)	120	≈60/60	1 laboratory session			X		X		
Ray *et al*. (2006)	60	≈30/30	2 laboratory sessions					X		X
Kelemen and Kaighobadi (2007)	120	≈60/60	1 laboratory session			X				X
Lindsey *et al*. (2007)	Not reported	Not reported	1 laboratory session							X
McClernon *et al*. (2007)	20	20/16	4 weeks					X		X
Kelemen (2008)	103	52/52	1 laboratory session		X		X			X
Attwood *et al*. (2009)	44	≈22/22	1 laboratory session							X
Brody *et al*. (2009a)	15	≈10/10	1 or 2 laboratory sessions		X					X
Brody *et al*. (2009b)	62	16/16	1 laboratory session							X
King *et al*. (2009)	42	42/42	2 laboratory sessions					X		
Rose *et al*. (2010)	20	20/20	2 laboratory sessions				X			X

10.4.4. Safety Conclusions from Clinical Trials

Like conventional cigarettes, X-22 cigarettes are toxic and pose health risks to subjects, namely cancer, cardiovascular disease, respiratory disease and reproductive effects.[3] However, previous clinical studies (see Section 10.2) have found that, when smoking very low nicotine cigarettes, smokers generally consume fewer cigarettes per day than when they smoke their usual brand. It is therefore expected that the exposure of subjects to smoke components in the proposed IND trial will be no greater than if they did not participate and continued to smoke their usual brand.

Previous clinical studies have not found evidence that VLN cigarettes are any less safe than regular cigarettes. The above literature review indicates that adverse events, vital signs measurements, and CO exposure are similar after smoking regular versus VLN cigarettes. It has been suggested that nicotine may mitigate some of the negative effects of other cigarette smoke components based on *in vivo* and *in vitro* laboratory studies[83,51]; however, it is expected that this theoretical risk will be mitigated by the planned short term exposure to the product in confirmed smokers and offset by the long-term benefits of increased smoking cessation rates.

It is not expected that the addition of menthol to X-22 cigarettes will have an adverse effect on the safety of this product.

The patient population evaluated in Study Vector-201 was similar to the target patient population for the X-22 development program; Study Vector-201 utilized Quest 3 cigarettes, which contain the same tobacco that is used in X-22 cigarettes; and Study Vector-201 was conducted under and IND according to GCPs. Therefore, for the purposes of AE reporting in clinical trials, the AEs reported by subjects receiving Quest cigarettes (ie, study Groups 1 and 2) during Study Vector-201 in ≥ 5% of subjects in either group, as shown below in Table 12-1 will be considered as "expected" during clinical trials of X-22 Smoking Cessation Product.

Table 10-9 Adverse Events Reported in Study Vector-201 in ≥5% of Subjects in Groups that Received Quest Cigarettes (Groups 1 or 2)

System Organ Class **Preferred Term**	**Group 1 (N=116) n (%)**	**Group 2 (N=116) n (%)**
Gastrointestinal Disorders		
Nausea	8 (7%)	7 (6%)
General Disorders and Administration Site Conditions		
Application Site Pruritus	8 (7%)	1 (<1%)

System Organ Class **Preferred Term**	**Group 1** **(N=116)** **n (%)**	**Group 2** **(N=116)** **n (%)**
Infections and Infestations		
Back Pain	4 (3%)	6 (5%)
Nervous System Disorders		
Headache	7 (6%)	12 (10%)
Psychiatric Disorders		
Insomnia	15 (13%)	6 (5%)
Anxiety	8 (7%)	2 (2%)
Irritability	7 (6%)	2 (2%)

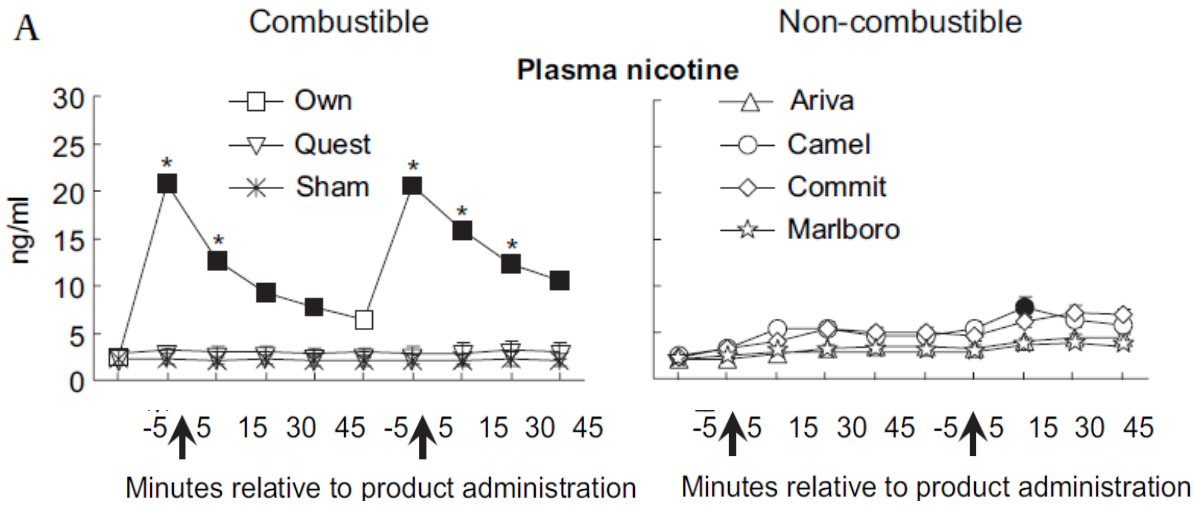
Source: CSR for Study Vector-201, Appendix A, Table S33

10.5. Clinical Pharmacokinetics

The clinical pharmacokinetics of the components of smoke from X-22 cigarettes have not yet been systematically evaluated. However, at least 2 studies have evaluated exposure to components of Quest 3 cigarettes and information is available regarding the pharmacology and pharmacokinetics of menthol. These data are provided in the subsections below.

10.5.1. Quest 3 Cigarettes

A study conducted by Cobb *et al*[63] compared blood nicotine values after the use of 7 different noncombustible PREPs (Ariva compressed tobacco tablet, Camel Snus, Marlboro Snus, and Commit® 2 mg Nicotine Lozenge) and combustible tobacco products (Subjects' OWN brand, SHAM [puffing on an unlit cigarette], and Quest 3 [nicotine yield ≤0.05 mg]). Nicotine levels reported from this study are provided in Figure 10-18, below. These data confirm that smoking cigarettes made with the VLN tobacco used in X-22 cigarettes does not result in appreciable increases in plasma nicotine.



Mean data (±SEM) for plasma nicotine. Arrows indicate product administration. Filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN brand mean relative to all non-combustible product means at that time point (<0.05, Tukey's Honestly Significant Difference)

Figure 10-18 Plasma Nicotine Levels Reported by Cobb *et al*, 2010

Hatsukami *et al*[55] (see also Section 10.2.1.2) measured levels of various biomarkers of smoking exposure in subjects who smoked Quest 2 (nicotine yield 0.30 mg) or Quest 3 (nicotine yield ≤0.05 mg) cigarettes, or who did not smoke but used 4 mg nicotine lozenges. Biomarkers of tobacco toxicant exposure measures included:

- Urinary cotinine plus cotinine–glucuronide (total cotinine), a direct measure of nicotine exposure.
- Urinary 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanol and its glucuronides (total NNAL), metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.
- Urinary N_-nitrosonornicotine and its glucuronide (total NNN), metabolites of the tobacco-specific carcinogen N_-nitrosonornicotine.
- Urinary 1-hydroxypyrene and its glucuronide and sulfate (total 1-HOP), a metabolite of pyrene which is an accepted biomarker for uptake of carcinogenic polycyclic aromatic hydrocarbons.
- Urinary 3- hydroxypropylmercapturic acid (3-HPMA), a metabolite of the toxicant, acrolein.
- Sphenylmercapturic acid (S-PMA), a metabolite of the human leukemogen, benzene.

These biomarkers reflect exposure to particulate or smoke constituents in cigarettes. 1-HOP, 3-HPMA, and S-PMA are influenced by factors other than tobacco, while total cotinine, total NNAL and total NNN are tobacco-specific. Results of these measures at baseline, Week 2, and Week 6 are shown in Table 10-10. Mean levels of all biomarkers measured decreased relative to baseline in the Quest 3 VLN cigarette group and mean levels of nearly all biomarkers (all except S-PMA at 2 weeks) were lower in the Quest 3 VLN cigarette, group than in the Quest 2, 0.30 mg nicotine cigarette, group at Weeks 2 and 6.

Table 10-10 Geometric Means of Biomarkers After Exposure to 0.6 mg or ≤0.05 mg Nicotine Cigarettes or Nicotine Lozenges at Baseline and After 2 and 6 Weeks of Treatment Published by Hatsukami *et al* 2010

	Geometric mean (95% confidence interval)		
Biomarkers	*Baseline*	*Week 2*	*Week 6*
Total cotinine[1]			
0.3 mg cigarettes	4057 (3323, 4952)	2150 (1696, 2725)*	2093 (1611, 2719)*[a]
0.05 mg cigarettes	4216 (3492, 5090)	278 (174, 442)*	188 (111, 319)*[b]
Nicotine lozenge	3917 (3399, 4514)	2291 (1708, 3073)*	2154 (1312, 3536)*[a]
Total NNAL[2]			
0.3 mg cigarettes	0.96 (0.73, 1.26)	0.54 (0.41, 0.69)*	0.47 (0.30, 0.73)*[a]
0.05 mg cigarettes	0.92 (0.70, 1.21)	0.34 (0.20, 0.57)*	0.20 (0.11, 0.34)*[b]
Nicotine lozenge	1.06 (0.84, 1.35)	0.24 (0.18, 0.32)*	0.14 (0.07, 0.26)*[b]
Total NNN[2]			
0.3 mg cigarettes	0.10 (0.06, 0.16)	0.09 (0.06, 0.14)	0.06 (0.04, 0.10)[a]
0.05 mg cigarettes	0.09 (0.05, 0.15)	0.06 (0.04, 0.11)	0.03 (0.02, 0.07)*[ab]
Nicotine lozenge	0.08 (0.05, 0.12)	0.02 (0.01, 0.04)*	0.02 (0.01, 0.04)*[b]
Total 1-HOP[2]			
0.3 mg cigarettes	0.84 (0.70, 1.02)	0.95 (0.58, 1.53)	0.73 (0.59, 0.90)[a]
0.05 mg cigarettes	0.89 (0.71, 1.12)	0.75 (0.56, 1.01)	0.57 (0.42, 0.78)*[a]
Nicotine lozenge	0.94 (0.71, 1.24)	0.40 (0.29, 0.56)*	0.34 (0.21, 0.57)*[b]
3-HPMA[2]			
0.3 mg cigarettes	3662 (2868, 4674)	2838 (2226, 3619)	2732 (2110, 3537)[a]
0.05 mg cigarettes	3320 (2667, 4134)	1639 (1215, 2211)*	1453 (1039, 2032)*[b]
Nicotine lozenge	3445 (2539, 4673)	911 (670, 1239)*	1062 (749, 1508)*[b]
S-PMA[2]			
0.3 mg cigarettes	2.21 (1.54, 3.18)	1.30 (0.88, 1.92)*	1.35 (0.94, 1.93)*[a]
0.05 mg cigarettes	2.46 (1.68, 3.62)	1.54 (1.03, 2.31)*	0.76 (0.48, 1.20)*[b]
Nicotine lozenge	2.69 (1.95, 3.72)	0.33 (0.22, 0.49)*	0.48 (0.30, 0.78)*[b]

[1]ng/ml.
[2]pmol/mg creatinine.
*$P < 0.05$ at that visit compared to baseline (within-group comparison). Groups with different letters were significantly different ($P < 0.05$) at the week 6 treatment visit (between-group comparison). For example, total cotinine is significantly different between the ≤0.05 mg cigarette group with 0.3 mg cigarette group and with nicotine lozenge group, but the 0.3 mg cigarette group is not significantly different from the nicotine lozenge group. Total NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides; total NNN: *N*_-nitrosonornicotine and its glucuronide; total 1-HOP: 1-hydroxypyrene and its glucuronide and sulfate; 3-HPMA: 3-hydroxypropylmercapturic acid; *S*-PMA: *S*-phenylmercapturic acid.

Data from the 2 studies presented above indicate that subjects who smoke cigarettes containing VLN tobacco are at no greater risk for exposure to nicotine (as measured in both plasma and urine) and several other substances than smokers of regular cigarettes, and smoking VLN tobacco may actual lessen exposure to these agents.

It should be noted, however, that if a VLN cigarette contains less than 1 mg of nicotine in the filler, the upper limit on how much nicotine could be inhaled by any smoker would be a small fraction of 1 mg. The bioavailability of nicotine from a cigarette varies widely with smoking behavior, but is typically about 12%, with a maximum of about 40%[95,96], so a smoker would be expected to take in 0.4 mg or less nicotine per cigarette, even if the cigarette was smoked more intensely.

10.5.2. Menthol

The information in this section was obtained from the 2011 Tobacco Products Scientific Advisory Committee's (TPSAC) "Draft Report and Recommendations on Menthol in Cigarettes".[34]

Pharmacology of Menthol in Cigarettes

With repeated or prolonged administration, menthol is known to cause desensitization to its own cooling and irritant effects. Menthol is also reported to reduce sensitivity to noxious chemicals, including nicotine. The irritating effects of nicotine on the airway are mediated by activation of nicotinic cholinergic receptors and TRPA1. Menthol did however reduce burning pain both in baseline and nicotine conditions. Higher levels of nicotine reduced the subjects' ability to discriminate dose-related odor and cooling effects of menthol compared to lower nicotine levels. While both menthol and nicotine have the potential to desensitize responses with repeated exposure, a study comparing olfactory thresholds for menthol and nicotine in smokers and non-smokers found the smokers had a much higher olfactory threshold for nicotine but no difference in threshold for menthol. The same was seen in both menthol and non-menthol smokers. Thus the effects of menthol are persistent in smokers.

Pharmacokinetics of Menthol in Cigarettes

Menthol moves from cigarette smoke into the lungs and then into the bloodstream. Smokers systemically absorb an average of 5–20 percent of the menthol in a menthol cigarette, depending on the extent of ventilation. For a cigarette containing 3 mg of menthol (0.3 percent), a smoker of 20 cigarettes per day is exposed to an average systemic dose of 12.5 mg menthol per day.

Once it enters the general circulation, menthol is rapidly metabolized, making it difficult to measure free menthol in the blood or urine. Menthol is metabolized primarily through glucuronidation, a process that takes place in the liver to detoxify substances, and through

oxidation, which also takes place in the liver. Glucuronidation primarily is driven by the liver enzyme UDP-glucuronosyl transferase 1A4. The result of this process is a compound called menthol glucuronide. Oxidation of menthol to hydroxylated metabolites has been observed in studies in rats. In humans, approximately 50% of an oral dose of menthol is excreted in the urine as menthol glucuronide. The half-life of menthol glucuronide after oral menthol dosing is about 50 minutes in plasma and 74 minutes in urine, although there appears to be a longer terminal half-life, most likely due to the slow release of the highly lipid-soluble menthol from body tissues and/or due to enterohepatic recirculation. It is difficult to do pharmacokinetic studies with inhaled menthol because the dose absorbed cannot be known with certainty. Urine menthol glucuronide concentrations have been measured in a cross-sectional study of smokers of menthol and non-menthol cigarettes. On average, menthol levels are higher in menthol smokers, but many non-menthol smokers also have high menthol levels due to consumption of menthol-containing foods.

While free menthol concentrations are quite low in blood, they are high in tobacco smoke. As a result, menthol concentrations will be high in the mouth, throat and lungs. Estimating concentrations in smoke is important to assess the plausibility that menthol has effects on sensory nerves and possible drug metabolism in the upper and lower airways in relation to concentrations that have effects in animals or cell preparations. Assuming that a menthol cigarette delivers 0.8 mg of menthol in smoke and that a smoker takes 8 puffs on a cigarette, the menthol per puff is 0.1 mg. Assuming that all of the menthol in a puff is absorbed and that the inhalation volume associated with one puff (puff volume plus air) is 800 ml, the concentration of menthol would be 1250 μg/L, which would be 8.0 μM/L. There is uncertainty about the partition of menthol between smoke and lung tissue, but this gives some rough approximation about what levels might act in the lungs, where there are drug metabolizing enzymes. Concentrations could be considerably higher in the mouth and throat, before the inhaled smoke is fully diluted with the fresh air inhaled with the smoke.

Particulate Matter in the Smoke of Mentholated Cigarettes

Several studies have shown that menthol increases the amount of tar and fine particles in cigarette smoke. The mechanism of increased particle formation was speculated to be greater transfer of the additive materials to the particle phase of the smoke compared to tobacco constituents. Increased particulate matter in smoke is of concern because particulates are associated with greater morbidity and mortality from cardiovascular disease and are suspected to increase the risk of sudden cardiac death. The smoke generated from cigarettes to which menthol was added also delivered higher levels of formaldehyde, and lead, both tobacco smoke toxicants, compared to smoke from the control cigarettes.

10.6. Conclusions from Previous Clinical Trials

- Previous clinical studies have not determined any significant differences in the overall safety profile of VLN cigarettes vs regular cigarettes.

- Previous clinical studies have found that, when smoking VLN cigarettes, smokers generally consume fewer cigarettes per day than when they smoke their usual brand.

- Although there has been concern that when smokers switch from their usual brand to VLN nicotine cigarettes, compensatory increases in smoking may occur, when switching to ≤0.05 mg nicotine cigarettes compensatory smoking has not been substantiated.

- Short term use of ≤0.05 mg nicotine cigarettes for 2 weeks is less effective than longer term use over 6 weeks.

- Although some studies have found significant efficacy with concomitant use of NRT, especially when the NRT was begun at least 2 weeks before the quit date, another study has found that ≤0.05 mg VLN cigarettes alone are quite effective, resulting in a 4-week continuous abstinence rate of 43% and a 6-week post-treatment point prevalence rate of 47%.

- Smoking ≤0.05 mg nicotine cigarettes for at least 6 weeks alone also seems to be more effective that using a step-down approach (e.g., 2 weeks of 0.6 mg nicotine cigarettes, followed by 2 weeks of 0.3 mg nicotine cigarettes, followed by 2 weeks of 0.06 mg nicotine cigarettes).

- Numerous studies have found that acute withdrawal effects and cravings after short term smoking abstinence, as evaluated in a laboratory setting, are decreased as effectively or nearly as effectively with VLN cigarettes as with regular cigarettes.

- Smoking VLN cigarettes was more effective than inhaled nicotine at relieving cravings and other smoking withdrawal symptoms.

- During a longer term study of smoking cessation, smoking VLN cigarettes were associated with less withdrawal effects compared to 4-mg nicotine lozenges.

- Evidence does not indicate increased disease risks in smokers of menthol cigarettes compared to non-menthol cigarettes; therefore, it is not expected that the addition of menthol to X-22 cigarettes will have an adverse effect on the safety of this product.

11. MARKETING EXPERIENCE

X-22 Smoking Cessation Product has not previously been marketed or studied anywhere in the world.

12. SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product in the form of a cigarette. Like conventional cigarettes, X-22 cigarettes consist of filler, filter, and wrapper components. X-22 cigarettes contain menthol, (b) (4) . The menthol is included in part to compensate for the reduced sensory impact of the cigarettes in the mouth and throat due to the very low nicotine content.

The filler in X-22 cigarettes, like that in conventional cigarettes, is comprised primarily of cured tobacco leaf and does not contain non-tobacco plant material (other than a relatively small amount present in some of the additives and flavorings included in the filler). The filler of X-22 cigarettes contains a proprietary VLN tobacco variety, Vector 21-41, which has very low levels of nicotine due to genetic modification of the nicotine biosynthesis pathway in the plant. X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of less than 1 mg/g and a machine smoking yield of ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes.

All processing of tobacco is carried out by processors of conventional tobacco. All manufacturing of cigarette components and cigarettes is carried out by manufacturers of conventional cigarette components and cigarettes.

12.1. Indication

X-22 Smoking Cessation Product is being developed for the indication of smoking cessation. The product is intended to reduce cravings for cigarettes and increase the chances of successfully quitting smoking.

12.2. Summary of Available Safety Data

Like conventional cigarettes, X-22 cigarettes are toxic and pose health risks to subjects, namely cancer, cardiovascular disease, respiratory disease and reproductive effects[3]. All X-22 cigarettes and active control cigarette packs are labelled with "Surgeon General's Warning: Cigarette Smoke Contains Carbon Monoxide."

Although numerous studies of cigarettes containing Vector 21-41 tobacco have been published (see Section 10.2), most of these publications focused on efficacy or subjective effects and did not address safety. The primary source of safety information for VLN cigarettes is a study conducted by Vector Tobacco, Inc. under IND 69,185. This study and others found no evidence that VLN cigarettes are any less safe than regular cigarettes. The above literature review indicates that adverse events, vital signs measurements, and CO

exposure are similar after smoking regular versus VLN cigarettes. It has been suggested that nicotine may mitigate some of the negative effects of other cigarette smoke components based on *in vivo* and *in vitro* laboratory studies[83,51]; however, it is expected that this theoretical risk will be mitigated by the planned short term exposure to the product and offset by the long-term benefits of increased smoking cessation rates.

The patient population evaluated in Study Vector-201 was similar to the target patient population for the X-22 Smoking Cessation Product development program; Study Vector-201 utilized Quest 3 cigarettes, which contain the same tobacco filler that is used in X-22 cigarettes; and Study Vector-201 was conducted under an IND according to GCPs. Therefore, for the purposes of AE reporting in clinical trials, the AEs reported by subjects receiving Quest cigarettes (ie, study Groups 1 and 2) during Study Vector-201 in ≥ 5% of subjects in either group, as shown below in Table 12-1 will be considered as "expected" during clinical trials of X-22 Smoking Cessation Product.

Table 12-1 Adverse Events Reported in Study Vector-201 in ≥5% of Subjects in Groups that Received Quest Cigarettes (Groups 1 or 2)

System Organ Class **Preferred Term**	**Group 1 (N=116) n (%)**	**Group 2 (N=116) n (%)**
Gastrointestinal Disorders		
Nausea	8 (7%)	7 (6%)
General Disorders and Administration Site Conditions		
Application Site Pruritus	8 (7%)	1 (<1%)
Infections and Infestations		
Back Pain	4 (3%)	6 (5%)
Nervous System Disorders		
Headache	7 (6%)	12 (10%)
Psychiatric Disorders		
Insomnia	15 (13%)	6 (5%)
Anxiety	8 (7%)	2 (2%)
Irritability	7 (6%)	2 (2%)

Source: CSR for Study Vector-201, Appendix A, Table S33

Results of a smoke composition comparison study show that the smoke from X-22 cigarettes contains no novel toxic compound(s) and that human subjects who inhale smoke from X-22 cigarettes will not be exposed to greater amounts of known toxic compound(s) than when they inhale smoke from standard US cigarette brands.

Previous clinical studies (see Section 10.2) have found that, when smoking VLN cigarettes, smokers generally consume fewer cigarettes per day than when they smoke their usual brand. It is therefore expected that the exposure of subjects to smoke components in the proposed IND trial will be no greater than if they did not participate and continued to smoke their usual brand.

Based on the body of information presented in this document, it is reasonable to conclude that X-22 Smoking Cessation Product poses no new hazard or health risk beyond the risks known for conventional cigarettes and that, under the conditions of usage proposed in the clinical trial, X-22 cigarettes should not pose any additional risk to subjects in the study beyond those already know to be attributable to smoking conventional cigarettes.

12.3. Contraindications

Pregnancy and Lactation

Because cigarette smoking has been shown to be harmful to the fetus, subjects who are pregnant or lactating will not be permitted to enroll in any clinical studies of X-22 Smoking Cessation Product. The use of cigarettes, and nicotine in particular, during pregnancy increases the risk of low birth weight, prematurity, spontaneous abortion, and perinatal mortality in humans.

12.4. Overdosage

Although nicotine exists in only very low amounts in X-22 cigarettes, the active control cigarettes in X-22 clinical trials have a nicotine content comparable to regular cigarettes. The acute toxicity of nicotine brings about dizziness, nausea, vomiting, headache, and diarrhea. More severe intoxication can occur, resulting in death, due to paralysis of respiratory muscles.

12.5. Dosage and Administration

During the treatment period of clinical studies, subjects will be permitted to smoke study cigarettes *ad libitum* until the protocol-specified quit smoking date.

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22nd Century Limited, LLC

CLINICAL STUDY PROTOCOL

Study Title:	A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid
Sponsor:	22nd Century Limited, LLC 8201 Main Street, Suite 6 Williamsville, NY 14221
IND No.:	103,589
Protocol Number:	X-22-201
22nd Century Medical Monitor:	(b) (4) Telephone: (b) (4), (b) (6) e-mail: (b) (4), (b) (6)
22nd Century Limited, LLC Vice President, Research & Development	Michael R. Moynihan, PhD Telephone: (b) (6) Fax: (b) (6)
Protocol Version/Date:	Final / 20 June 2011

CONFIDENTIALITY STATEMENT

The information contained in this document, particularly unpublished data, is the property or under control of 22nd Century Limited, LLC, and is provided to you in confidence as an investigator, potential investigator, or consultant, for review by you, your staff, and an applicable Institutional Review Board or Independent Ethics Committee. The information is only to be used by you in connection with authorized clinical studies of the investigational product described in the protocol. You will not disclose any of the information to others without written authorization from 22nd Century Limited, LLC, except to the extent necessary to obtain informed consent from those persons to whom the product may be administered.

22nd Century Limited, LLC
8201 Main Street, Suite 6
Williamsville, NY 14221

STUDY ACKNOWLEDGEMENT

A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid

This protocol has been approved by 22nd Century Limited, LLC. The following signatures document this approval.

(b) (6)

Date: 6/21/11

Michael R. Moynihan, PhD
Vice President, Research & Development

(b) (6)

Date: June 21, 2011

Joseph Pandolfino, MBA
CEO

INVESTIGATOR STATEMENT

I agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by 22nd Century Limited, LLC.
- Not to implement any changes to the protocol without written agreement from 22nd Century Limited, LLC and prior review and written approval from the Institutional Review Board (IRB) except where necessary to eliminate an immediate hazard to subjects.
- That I am thoroughly familiar with the appropriate use of the investigational product, as described in this protocol and any other information provided by 22nd Century Limited, LLC including, but not limited to, the current Investigator's Brochure (IB).
- That I am aware of, and will comply with, good clinical practices (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the 22nd Century Limited, LLC investigational product and of their study-related duties and functions as described in the protocol.

Signature: _____ Date: _____

Name (print): _____
Principal Investigator

Site Number: _____

2. PROTOCOL SYNOPSIS

22nd Century Limited, LLC
8201 Main Street, Suite 6
Williamsville, NY 14221

Title of Study:	A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid
IND Number:	103,589
Study Centers Planned:	3 centers in the United States
Objectives:	The primary objective of this study is to evaluate the efficacy of X-22 as a smoking cessation aid. Secondary objectives of the study include: • To assess the effect of X-22 on abstinence at 2 and 3 months after the quit date, • To assess withdrawal symptoms, • To evaluate the effect of X-22 on cigarette consumption prior to the quit date, and • To evaluate the tolerability of X-22 as a smoking cessation aid. Exploratory objectives include: • To evaluate other factors that may have an influence on the efficacy of X-22 as a smoking cessation aid, including subject gender, results of a Sensory Questionnaire prior to the quit date, and results of the Fagerström Test of Nicotine Dependence prior to the quit date. • To compare nicotine use between groups over time.

Study Design:	This will be a. prospective, randomized, multicenter, double blind, parallel, active controlled, Phase II clinical trial in which a total of 216 healthy smokers will be enrolled and followed over approximately 19 weeks at a maximum of 3 sites. Healthy smokers who plan to quit smoking will be screened for eligibility at Visit 1 (Screening Phase). Screening will include documentation of medical/smoking history, physical examination, clinical laboratory tests, electrocardiogram, urine pregnancy test (in women of child-bearing potential), drug screen, exhaled CO, saliva cotinine level, and the completion of 3 questionnaires (Sensory Questionnaire, Minnesota Nicotine Withdrawal Scale, and the Fagerström Test of Nicotine Dependence). All subjects will receive behavioral support through approximately 15-minute individual counseling sessions at Visits 4, 5, 6, and 9, including distribution of printed materials. Subjects will continue to smoke their usual brand *ad libitum* during Week 1. One week following Screening, at Visit 2 (beginning of the Treatment Phase), eligible subjects will be randomized in a 1:1 ratio to 1 of the 2 treatment arms: • Group 1: X-22 Smoking Cessation Product • Group 2: Active Control Cigarettes The randomization will be stratified by gender. At Visit 2, subjects will switch from their usual brand cigarettes to X-22 cigarettes (Group 1) or blinded active control cigarettes (Group 2) and will smoke the assigned study cigarettes *ad libitum* for 6 weeks prior to the target quit date. Visit 5/Week 6 is considered the "quit date" (quit all smoking) for all study subjects. After randomization, during the Treatment and Abstinence Phases, subjects will return to the study center at 2-week intervals for 10 weeks (Visits 2 to 7), and will receive a telephone call from the site between visits at Weeks 1, 3, 5, 7, and 9. During the Follow-up Phase, subjects will receive a follow-up telephone call at Week 14. At the Week 14 follow-up telephone call, subjects who report that they have not smoked in the past 7 days will be asked to return to the study site within 1 week for Visit 8. At Week 18, all subjects will return to the site for Visit 9, regardless of smoking status. Subjects will receive information regarding the use of approved smoking cessation pharmacotherapies during their Visit 9 counseling session. Cigarette consumption will be evaluated using self-report diaries and measurement of exhaled CO and cotinine concentrations. Withdrawal symptoms will be assessed using The Minnesota Nicotine Withdrawal Scale at each study visit. The Sensory Questionnaire and the Fagerström Test of Nicotine Dependence will be administered at study Visits 1 to 5. Safety will be assessed by evaluation of adverse events, physical examinations, clinical laboratory tests, and measurement of heart rate, blood pressure, and body weight at each study visit.

Number of Subjects Planned:	216
Target Population:	Healthy adult smokers who plan to quit smoking.
Duration of Treatment:	This study will consist of a 1-week Screening Phase, a 6-week Treatment Phase, a 4-week Abstinence Phase, and an 8-week Follow-up Phase, for a total of approximately 19 weeks of study enrollment.
Diagnosis and Main Eligibility Criteria:	Inclusion Criteria: Subjects must meet *all* of the following inclusion criteria to be eligible for participation in this study. 1. Subjects must be considered by the investigator to be in general good health and between 18 and 65 years of age, inclusive. 2. Subjects must have a history of smoking at least 10 cigarettes per day for at least 1 year, with less than 3 months of abstinence during the past year, and a continuous smoking history for at least 3 months prior to study entry. 3. Subjects must indicate that they plan to quit smoking within the next 3 months. 4. Subjects must have a CO measurement of ≥ 10 ppm at baseline. 5. Subjects must be willing and able to return for scheduled follow-up examinations for a total of 5 months. 6. Female subjects must have been postmenopausal for the previous 6 months or be surgically sterile, or must have a negative pregnancy test and use one of the following acceptable methods of birth control for the duration of the study and for at least 3 months prior to screening: a. abstinence, meaning a total lack of sexual activity, b. oral contraceptives (“the pill”), c. contraceptive injections, d. intrauterine device, e. double-barrier method (diaphragm or condom plus spermicidal cream), f. contraceptive patch, hormonal implant, hormonal vaginal ring, or g. male partner sterilization at least 3 months prior to screening. 7. Subjects must sign and be given a copy of the written Informed Consent form. 8. Subjects must be able to read, understand and complete the questionnaires independently.

Diagnosis and Main Eligibility Criteria (continued):

Exclusion Criteria

Subjects who meet *any* of the following exclusion criteria are not to be enrolled in this study.

1. Subjects who have a household member who is already enrolled in this trial.
2. Subjects who have attempted to quit smoking using one or more FDA-approved smoking cessation products (eg, NRT including nicotine gum, nicotine lozenges, nicotine patches, nicotine nasal spray, or nicotine inhaler; Zyban [bupropion]; or Chantix [varenicline]) for longer than 1 week in the past 3 months.
3. Subjects who have been enrolled in another structured smoking cessation program (e.g., behavioral modification, hypnosis, acupuncture, or other alternative therapies) in the last 30 days.
4. Subjects who have been in another smoking cessation trial in the past 6 months.
5. Subjects who regularly use (greater than once per month) tobacco-based products other than cigarettes (eg, chew, snuff, snus, shisha, etc.).
6. Subjects with a history of coronary artery disease, myocardial infarction, stroke, chronic obstructive pulmonary disease, or other significant pulmonary diseases. Controlled hypertensive subjects and mild asthmatics whose disease is controlled only with an as-needed inhaled beta agonist are acceptable.
7. Subjects with a history of cancer within the past 60 months other than basal cell carcinoma.
8. Subjects with a history of thromboembolic disease or taking warfin or any other anticoagulant, or anti-platelet drug such as clopidogrel (Plavix®) or pentoxifylline (Trental®). Low dose aspirin therapy is acceptable.
9. Subjects with screening laboratory abnormalities that are considered by the investigator to be clinically significant.
10. Subjects with a body mass index (BMI) >35.
11. Subjects with poorly controlled diabetes or insulin-dependent diabetes.
12. Subjects with other known serious pathophysiology or topical or systemic disorders of any kind that would confound the results of the study.
13. Subjects with screening ECG abnormalities that are considered by the investigator to be clinically significant.
14. Subjects who have used any illegal drug in the past 3 months.
15. Subjects who have a positive urine drug screening result.
16. Subjects who are pregnant or lactating, or who plan to become pregnant during the course of the study.

Diagnosis and Main Eligibility Criteria (continued):	17. Subjects participating in any other clinical trial of an investigational drug or device during the time of this clinical investigation or within 30 days prior to Screening visit. 18. Subjects taking anti-depressants, anti-psychotics, clonidine, or any of the classes of drugs listed in the RapidCHECK® 12 Panel in the last 60 days (see Section 12.1.1). 19. Subjects with systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg. 20. Subjects consuming an average of 3 or more drinks of alcohol per day. 21. Subjects consuming greater than an average of 2 packs of cigarettes per day.
Test Product, Dose, and Mode of Administration:	The X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product consisting of very low nicotine (VLN) cigarettes. X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of less than 1 mg/g and a machine smoking yield of ≤ 0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes. The X-22 cigarettes contain menthol, (b) (4) Investigational product will be dispensed at study Visits 2, 3, and 4. At Visit 2, the number of cigarettes dispensed will be approximately equal to the number of usual brand cigarettes the subject has reported smoking in a typical 2-week period plus an overage of 20%. The number of cigarettes dispensed may be adjusted at Visits 3 and 4 for up to a maximum of 11 cartons (110 packs of 20 cigarettes) for the 6-week Treatment Phase.
Reference Therapy, Dose, and Mode of Administration:	The Active Control cigarette is identical to the X-22 cigarette except for the filler, which has a nicotine content similar to that of a conventional "light" cigarette with a machine smoking yield of approximately 0.7 mg nicotine per cigarette. The active control cigarettes, like the X-22 cigarettes, contain menthol. The active control cigarettes will be dispensed under blinded conditions in the same manner as the X-22 cigarettes.

Criteria for Evaluation:	Efficacy Assessments: The primary efficacy measurement is the number of subjects with 4 weeks of continuous abstinence from smoking cigarettes (Visits 5-7) as assessed by subject diary and confirmed by exhaled CO. Secondary efficacy measurements include point prevalence smoking abstinence during the follow-up period (at Weeks 14 and 18); withdrawal symptoms as measured by the Minnesota Nicotine Withdrawal Scale at each study visit; the degree of dependence on smoking as measured at Screening and during the treatment phase using the Fagerström Test of Nicotine Dependence; results of a sensory questionnaire, which will be administered to obtain detailed information about the sensory properties of the X-22 cigarettes; and cotinine levels measured at each study visit to estimate nicotine use over time. Safety Assessments: All AEs spontaneously reported, elicited, or observed by the investigators will be recorded at each study visit. Heart rate, blood pressure and body weight will be measured at each study visit. Physical examinations and urine pregnancy tests will be conducted at Screening and Visit 7. Clinical laboratory tests will be conducted at Screening and Visit 5.
Statistical Methods:	The primary efficacy endpoint of the proportion of subjects achieving 4 weeks of abstinence from smoking following treatment will be compared using a Cochran-Mantel-Haenszel (CMH) test statistic stratified by gender. Secondary endpoints will be summarized by treatment group and assessment time. The assessment of between treatment difference on the secondary endpoints will be carried out using a generalized estimating equation (GEE) model or analysis of covariance (ANCOVA). The demographics and baseline characteristic will be included as covariates of interest. Severity of withdrawal symptoms will be compared between treatments using Chi-square test or Fisher's exact test. Safety endpoints will be analyzed by frequency of events/abnormalities for categorical values or descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) for continuous data by treatment group.

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4. LIST OF ABBREVIATIONS

° C	degrees Celsius
AE	adverse event
ANCOVA	analysis of covariance
CFR	Code of Federal Regulations
CI	confidence interval
CO	carbon monoxide
CRF	case report form(s)
CRO	Contract Research Organization
ECG	electrocardiogram
FDA	(U.S.) Food and Drug Administration
FTND	Fagerström Test of Nicotine Dependence
GCP	Good Clinical Practice (Guidelines)
GEE	generalized estimating equation (model)
HIPPA	Health Insurance Portability Accountability Act of 1996
IB	investigator brochure
ICH	International Conference on Harmonisation
IND	Investigational New Drug (application)
IRB	institutional review board
ITT	intent-to-treat (population)
IV	intravenous
mg	milligram(s)
mL	milliliter(s)
NDA	new drug application
NRT	nicotine replacement therapy
PP	per protocol (population)
RNC	reduced nicotine cigarette
SAE	serious adverse event
TEAE	treatment-emergent adverse event
UB	usual brand
VLN	very low nicotine (cigarette)

5. INTRODUCTION

5.1. Background

It has been well established that cigarette smoking is toxic and poses health risks to subjects, namely cancer, cardiovascular disease, respiratory disease and reproductive effects.[1] Cigarette smoking is a complex behavior that is sustained by the pharmacological properties of nicotine and reinforcing behavioral and sensory cues.[2,3,4] Currently available pharmacological approaches for smoking cessation in combination with counseling and education (maximal intervention) are effective in a minority of smokers, with the probability of initial success with all types of approved pharmacotherapy increased 1.5 to 2.5 fold compared to placebo.[5,6] Relapse is common following use of currently approved treatments, and 75 to 80 percent of smokers who receive treatment are unable to achieve long-term abstinence at 6 or 12 months. [6,7,8] Therefore, there is an important public health need to provide additional smoking cessation treatments, reduce relapse, and to provide alternative treatments for smokers who may not succeed with, or do not have access to, treatment that includes currently approved pharmacotherapies.

Cigarettes containing very low levels of nicotine consistently reduce craving for cigarettes and withdrawal in smokers.[9,10,11,12,13,14] In contrast to conventional low-yield ("light" or "ultra light") cigarettes that have reduced tar and nicotine yields as measured by smoking machines mainly due to smoke dilution, smoke from cigarettes containing very low levels of nicotine contains greatly reduced ratios of nicotine to other smoke components. The data from studies of the behavioral and pharmacodynamic properties of cigarettes that contain very low levels of nicotine indicate that a product that delivers the sensory and behavioral cues associated with cigarette use but does not deliver a satisfying amount of nicotine breaks the association of those cues with the contingent delivery of nicotine reinforcement and promotes extinction of smoke cues.

For further information regarding X-22 Smoking Cessation Product, please refer to the X-22 Investigator's Brochure.

5.2. X-22 Very Low Nicotine Cigarette

The X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product consisting of very low nicotine (VLN) cigarettes. X-22 cigarettes, like conventional cigarettes, consist of filler, filter, and wrapper components. X-22 cigarettes contain menthol,

(b) (4) The menthol is included in part to compensate for the reduced sensory impact of the cigarettes in the mouth and throat due to the very low nicotine content.

The filler in X-22 cigarettes, like that in conventional cigarettes, is comprised primarily of cured tobacco leaf and does not contain non-tobacco plant material (other than a relatively small amount present in some of the additives and flavorings included in the filler). A proprietary VLN tobacco variety, Vector 21-41, is used in the filler of X-22 cigarettes. All processing of tobacco is carried out by processors of conventional tobacco. All manufacturing of cigarette components and cigarettes is carried out by manufacturers of conventional cigarette components and cigarettes.

Like conventional cigarettes, X-22 cigarettes are toxic and pose health risks to subjects, namely cancer, cardiovascular disease, respiratory disease and reproductive effects. X-22 cigarettes are similar to conventional cigarettes except that the filler contains a proprietary VLN tobacco variety, Vector 21-41, which has very low levels of nicotine due to genetic modification of the nicotine biosynthesis pathway in the plant. X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of less than 1 mg/g and a machine smoking yield of ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes.

Except for the use of the VLN tobacco variety, the growing, curing and processing of tobacco for X-22 cigarettes are the same as for conventional cigarettes, and X-22 cigarettes have the same physical and chemical properties as conventional cigarettes, except for the reduction in nicotine and related compounds. The composition of smoke from X-22 cigarettes is chemically similar to smoke from conventional cigarettes.[15]

Quest® cigarettes, manufactured using the same VLN tobacco as X-22 cigarettes, were sold by Vector Tobacco Inc. from December 2002 to December 2007, and Quest menthol cigarettes were sold by Vector Tobacco Inc. from November 2003 through December 2007. Quest and Quest menthol cigarettes, each containing 3 levels of nicotine in the tobacco filler, resulted in the following nicotine yields per cigarette: Quest 1® (0.60 milligrams [mg]), Quest 2® (0.3 mg) and Quest 3® (≤ 0.05 mg). In addition to being sold commercially, Quest 3 cigarettes have been used in clinical studies, including trials evaluating their usefulness as an aid to smoking cessation, as described below in Section 5.3.

Vector-21-41 was developed at North Carolina State University (NCSU) in a research program funded by 22nd Century.

5.3. Smoking Cessation Trials of Very Low Nicotine Cigarettes

X-22 cigarettes have not previously been studied in humans; however, several clinical studies of Quest 3 VLN cigarettes for smoking cessation have been conducted in the past decade. Both X-22 cigarettes and Quest 3 contain the same VLN tobacco variety. Overall, results have shown that the use of VLN cigarettes is associated with consistently reduced craving for cigarettes, reduced withdrawal symptoms, and increase in smoking cessation rates compared to the use of nicotine replacement therapy (NRT) or reduced nicotine cigarettes (RNCs). Several recent studies are described below.

For further information refer to the Investigator's Brochure.

5.3.1. A Study of Quest 1, Quest 2, and Quest 3 in a Step-down Treatment Schedule with and without Nicotine Replacement Compared to a Conventional Cigarette

A randomized, double-blind, active controlled, parallel group, multi-center, Phase II clinical trial was conducted to evaluate the efficacy of reduced-nicotine cigarettes as a novel smoking cessation treatment under IND 69,185.[16] Treatment consisted of 6 weeks of smoking a series of cigarettes with progressively lower nicotine content (Quest 1, Quest 2, and Quest 3, with machine smoking yields of 0.6, 0.3, and ≤0.05 mg nicotine/cigarette, respectively), either in combination with nicotine patch therapy or placebo patches. Three hundred forty-six smokers of non-menthol cigarettes who were motivated to quit were randomized to one of 3 treatments:

- Group 1: Quest plus NRT patch,
- Group 2: Quest plus placebo patch, or
- Group 3: active control (conventional cigarette) plus NRT patch.

The primary endpoint was 4 weeks of continuous abstinence (Weeks 7-10), with follow-up at 3 and 6 months. Group 1 had a higher continuous abstinence during weeks 7-10 than Group 3, 32.8% vs. 21.9% ($p = 0.04$). Group 2 had an abstinence rate similar to that of Group 3, 16.4% vs. 21.9% ($p = 0.89$). Abstinence rates at the 3-month follow-up were 17.2%, 10.3%, and 14.9% in Groups 1, 2, and 3, respectively. At the 6-month follow-up, abstinence rates were 11.2%, 7.8%, 9.6% in Groups 1, 2, and 3, respectively. The differences in abstinence among groups were not statistically significant at 3 months and 6 months. Mean exhaled CO levels increased moderately compared to baseline after subjects smoked Quest 1 (Group 1, +1.5 ppm; Group 2, +2.5 ppm) and Quest 2 (Group1, +2.9 ppm; Group 2, +2.9

ppm), and was reduced compared to baseline after smoking Quest 3 (Group 1, -5.4 ppm; Group 2, -3.0 ppm). No serious adverse events (SAEs) were attributed to the investigational product and overall the incidence of adverse events (AEs) was similar between the treatment groups.

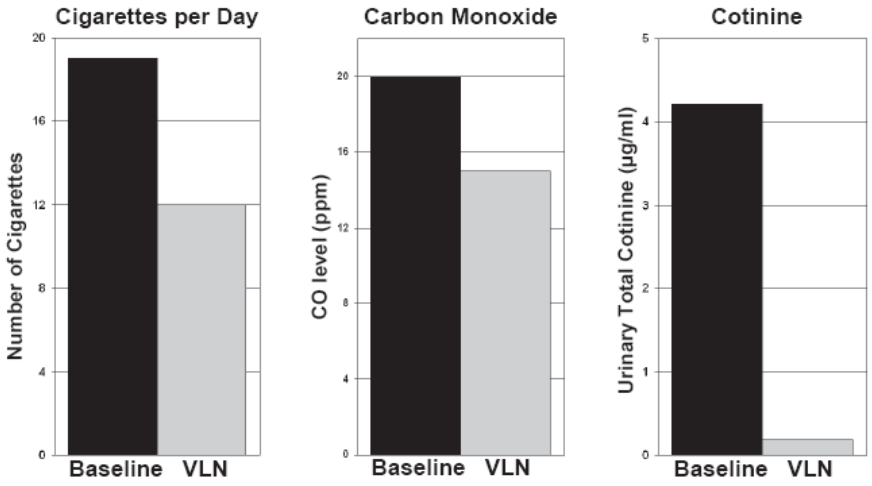
5.3.2. A Study of Quest 3 or Quest 2 Compared to Nicotine Lozenge

Another controlled cessation trial compared the effects of smoking VLN cigarettes or RNCs to the use of nicotine lozenges[17]. Smokers were randomly assigned to 1 of 3 treatment groups which were each administered for 6 weeks:

- Group 1: VLN Quest 3 cigarettes having a nicotine yield of ≤0.05 mg (n = 53)
- Group 2: reduced nicotine Quest 2 cigarettes having a nicotine yield of 0.3 mg (n=52)
- Group 3: 4 mg nicotine lozenge (n = 60)

Continuous abstinence 4-weeks post-treatment was 43% for the VLN cigarette group, 35% for the nicotine lozenge group, and 21% for the low nicotine cigarette group. Point abstinence rates at 6 weeks post-treatment were 47% for the VLN cigarette group, 37% for the nicotine lozenge group, and 23% for the low nicotine group. Unlike the Quest 2 cigarette, the Quest 3 VLN cigarette was not associated with compensatory smoking behaviors. Furthermore, Quest 3 VLN cigarette and nicotine lozenge were associated with reduced carcinogen exposure, nicotine dependence and product withdrawal scores. The Quest 3 VLN cigarette was associated with greater relief of withdrawal from usual brand cigarettes than the nicotine lozenge. The Quest 3 VLN cigarette led to a significantly higher rate of cessation than the Quest 2 cigarette and a similar rate as nicotine lozenge.

Patients who used the VLN Quest 3 cigarette over the 6-week treatment period significantly reduced their smoking as compared to their usual brand of cigarettes. The number of VLN cigarettes smoked per day on average decreased from 19 (the baseline number of cigarettes of the smoker's usual brand) to 12 by the end of the 6-week treatment period, even though participants in this clinical trial were instructed to smoke *ad libitum* (as many cigarettes as desired) during treatment. As depicted in Figure 5-1 below, carbon monoxide (CO) levels, an indicator of smoke exposure, significantly decreased from 20 parts per million (baseline) to 15 parts per million. Cotinine, a metabolite and biomarker of nicotine, significantly decreased from 4.2 micrograms/mL (baseline) to 0.2 micrograms/mL. All differences in the above three measurements were statistically significant ($p<0.05$).



Note: Figure created by 22nd Century from data published by Hatsukami et al, 2010

Figure 5-1 Number of Cigarettes Smoked and Carbon Monoxide and Cotinine Levels Published by Hatsukami et al, 2010

Additional biomarkers of smoke exposure were significantly reduced on average from baseline measurements (taken before the 6-week treatment period) in patients who used the Quest 3 VLN cigarette (see Figure 5-2).

BIOMARKER	DESCRIPTION	LEVEL IN PATIENTS (pmol/mg creatinine)		REDUCTION
		Baseline	VLN Cigarette	
NNAL	Metabolites of the tobacco-specific carcinogen NNK	0.92	0.2	78%
NNN	Metabolites of the tobacco-specific carcinogen NNN	0.09	0.03	67%
1-HOP	Metabolite of pyrene, a marker for uptake of carcinogenic polycyclic aromatic hydrocarbons	0.89	0.57	36%
3-HPMA	Metabolite of the smoke toxicant acrolein	3320	1453	56%
S-PMA	Metabolite of the carcinogen benzene	2.46	0.76	69%
	All differences were statistically significant (P<0.05).			

Note: Figure created by 22nd Century from data published by Hatsukami et al, 2010

Figure 5-2 Biomarkers of Smoke Exposure Published by Hatsukami et al, 2010

No significant differences were observed in biomarkers for cardiovascular risk between groups.

5.3.3. A Comparison of Quest 1 Without Nicotine Replacement Therapy Versus Quest 3 With Nicotine Replacement Therapy

In a randomized, controlled clinical trial conducted at Roswell Park Cancer Institute, Buffalo, New York, the effect of smoking a VLN cigarette in combination with a nicotine patch for 2 weeks prior to the quit date was investigated.[18] Ninety-eight adult heavy smokers (using 20 or more cigarettes/day) were randomized to 1 of 2 treatments:

- Group 1: 2 weeks of a VLN cigarette (Quest 3, nicotine yield ≤0.05 mg) and 21-mg nicotine patch before the quit date; or
- Group 2: RNC (Quest 1, nicotine yield 0.6 mg) during the 2 weeks before the quit date.

After the quit date, all subjects received counseling for smoking cessation and nicotine patch therapy for up to 8 weeks (4 weeks of 21-mg patches, 2 weeks of 14-mg patches, and 2 weeks of 7-mg patches). Group 1, which used VLN cigarettes and nicotine patch before quitting had lower combined craving scores during the 2 weeks before ($p = 0.001$) and after ($p = 0.013$) the quit date. Self-reported point prevalence of smoking abstinence at the 3- and 6-month follow-ups was higher in Group 1 (43% vs. 34% and 28% vs. 21%, respectively), although the difference was not statistically significant at the $p < 0.05$ level.

5.3.4. Study of Quest 3 With or Without Nicotine Replacement Therapy or With Quest 1 and Quest 2 in a Step-down Treatment Schedule

A small, unpublished study was carried out[19,20] comparing the following groups:

- Group 1: Quest 3 (nicotine yield ≤0.05 mg, tar yield 9 mg) with NRT
- Group 2: Quest 3 without NRT
- Group 3: Use of a series of cigarettes of decreasing nicotine content (Quest 1, nicotine yield 0.6 mg, Quest 2, nicotine yield 0.3 mg, and Quest 3, nicotine yield ≤0.05 mg)

During the first week of the study, subjects smoked only their usual brand of cigarette. From Weeks 2 through 5, subjects gradually reduced the amount of usual brand and increased the amount of Quest 3 smoked (Groups 1 and 2), or smoked cigarettes with decreasing amounts of nicotine per cigarette (Group 3). During Weeks 6 through 11, subjects were to smoke Quest 3 exclusively. During Week 12 through Week 14, subjects were gradually weaned off Quest 3, and subjects using NRT were weaned off of nicotine replacement. In Group 2, 33% of subjects achieved 4-week continuous abstinence. Group 3 attained a 13% abstinence rate. In Group 1 no subjects achieved abstinence.

5.4. Gender Differences in Smoking and Nicotine Effects

Previous research has found that the smoking behavior may be influenced by the gender of the subject; specifically, that women are influenced less by nicotine and more by other factors.[21] Although men and women do not differ in the physiological, cognitive, or psychomotor effects of nicotine, they may differ in their sensitivity to nicotine's reinforcing and rewarding effects.[22] This may be why women have more difficulty in quitting smoking in general.[21,23,24,25,26]

There is evidence that the use of NRT has less influence on long-term quit rates in women versus men.[21] West et al[27] found that women tended to do worse than men after use of NRT gum, patch, or nasal spray, but better than men when using an NRT inhaler as measured by 15-week abstinence rates in an uncontrolled open-label trial. Therefore, some formulations of NRT may be more effective in women than in men. There is also evidence that smoking cessation counseling may be more effective in women than in men.[24]

One approach in smoking cessation therapies is to provide substitutes that simulate the sensory effects of smoking. Standard counseling recommends strategies such as sucking on a straw or consuming carrot sticks or cinnamon sticks as substitutes for the motor effects of smoking.[21] However, these methods mimic only the motor effects of smoking, but not other sensory effects such as olfactory and taste stimuli and sensations to the throat. VLN cigarettes match almost all of the effects of smoking other than nicotine intake and therefore, may be particularly useful as a smoking cessation tool in women. This hypothesis is supported by Barrett[28], who found that women experienced greater relief of withdrawal-related cravings than men after smoking Quest cigarettes.

5.5. Information about Comparative Product

The Active Control cigarette is identical to the X-22 cigarette except for the filler, and has a conventional machine nicotine smoking yield of approximately 0.7 mg per cigarette. The Active Control cigarette, like the X-22 cigarette, contains menthol.

5.6. Rationale for the Current Study

There is an important public health need to provide additional smoking cessation treatments, reduce relapse, and to provide alternative treatments for smokers who may not succeed with, or do not have access to, treatment that includes NRT. Previous clinical studies have shown that the use of VLN cigarettes is associated with consistently reduced craving for cigarettes,

reduced withdrawal symptoms, and increase in smoking cessation compared to the use of NRT or RNCs. This proposed Phase II study will assess the magnitude of effectiveness that can be obtained with the X-22 Smoking Cessation Product. Because some previous studies have found differences between men and women in the response to nicotine and other factors affecting smoking, the treatment groups in this study will be stratified by gender. The results of this study will serve to optimize the design of the pivotal trials (Phase III), and provide objective data to support a decision regarding the development of the product concepts.

6. OBJECTIVES

The primary objective of this study is to evaluate the efficacy of X-22 as a smoking cessation aid.

Secondary objectives of the study include:

- To assess the effect of X-22 on abstinence at 2 and 3 months after the quit date.
- To assess withdrawal symptoms.
- To evaluate the effect of X-22 on cigarette consumption prior to the quit date.
- To evaluate the tolerability of X-22 as a smoking cessation aid.

Exploratory objectives include:

- To evaluate other factors that may have an influence on the efficacy of X-22 as a smoking cessation aid, including subject gender, results of a Sensory Questionnaire prior to the quit date, and results of the Fagerström Test of Nicotine Dependence prior to the quit date.
- To compare nicotine use between groups over time.

7. ENDPOINTS

7.1. Primary Efficacy Endpoint

The primary efficacy endpoint is 4 weeks of continuous abstinence (Weeks 7-10) assessed after treatment termination, at Visits 6 and 7.

7.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints include:

- 7-day point prevalence abstinence rates at 2 and 3 months following treatment termination.
- Withdrawal symptoms as measured by the Minnesota Nicotine Withdrawal Scale.
- Cigarette consumption during the treatment period.

7.3. Exploratory Endpoints

Exploratory endpoints will include:

- Results of the Sensory Questionnaire.
- Results of the Fagerström Test of Nicotine Dependence.
- Subjects' guess at Visit 7 regarding which treatment group they were assigned to.
- Cotinine levels at each study visit.

7.4. Safety Endpoints

Safety Endpoints will include:

- Incidence of treatment emergent adverse events (TEAEs)
- Physical examination results
- Vital signs measurements
- Clinical laboratory results

8. STUDY DESIGN

This will be a prospective, randomized, multicenter, double blind, parallel, active controlled, Phase II clinical trial in which a total of 216 healthy smokers will be enrolled and followed over approximately 19 weeks at a maximum of 3 sites.

Healthy smokers who plan to quit smoking will be screened for eligibility at Visit 1 (Screening Phase). Screening will include documentation of medical/smoking history, physical examination, clinical laboratory tests, electrocardiogram (ECG), urine pregnancy test (in women of child-bearing potential), drug screen, exhaled CO, saliva cotinine level, and the completion of 3 questionnaires (Sensory Questionnaire, Minnesota Nicotine Withdrawal Scale, and the Fagerström Test of Nicotine Dependence). All subjects will receive behavioral support through approximately 15-minute individual counseling sessions at Visits 4, 5, 6, and 9, including distribution of printed materials. Subjects will continue to smoke their usual brand *ad libitum* during Week 1.

One week following Screening, at Visit 2 (beginning of the Treatment Phase), eligible subjects will be randomized in a 1:1 ratio to 1 of the 2 treatment arms:

- Group 1: X-22 Smoking Cessation Product
- Group 2: Active Control Cigarettes

The randomization will be stratified by gender.

At Visit 2, subjects will switch from their usual brand cigarettes to X-22 cigarettes (Group 1) or blinded active control cigarettes (Group 2) and will smoke the assigned study cigarettes *ad libitum* for 6 weeks prior to the target quit date. Visit 5/Week 6 is considered the "quit date" (quit all smoking) for all study subjects.

After randomization, during the Treatment and Abstinence Phases, subjects will return to the study center at 2-week intervals for 10 weeks (Visits 2 to 7), and will receive a telephone call from the site between visits at Weeks 1, 3, 5, 7, and 9.

During the Follow-up Phase, subjects will receive a follow-up telephone call at Week 14. At the Week 14 follow-up telephone call, subjects who report that they have not smoked in the past 7 days will be asked to return to the study site within 1 week for Visit 8. At Week 18, all subjects will return to the site for Visit 9, regardless of smoking status. Subjects who fail to

quit smoking by the end of the study will receive information regarding the use of approved smoking cessation pharmacotherapies during their Visit 9 counseling session.

Cigarette consumption will be evaluated using self-report diaries and measurement of exhaled CO and cotinine concentration. Withdrawal symptoms will be assessed using The Minnesota Nicotine Withdrawal Scale at each study visit. The Sensory Questionnaire and the Fagerström Test of Nicotine Dependence will be administered at study Visits 1 to 5. Safety will be assessed by evaluation of AEs, physical examinations, clinical laboratory studies, concomitant medications, and measurement of heart rate, blood pressure, and body weight at each study visit.

9. SUBJECT POPULATION

9.1. Number of Subjects and Subject Selection

Two hundred sixteen smokers who plan to quit smoking, have provided informed consent for participation in this trial, who are capable of comprehending the nature of the study, and who are likely to comply with the visit schedule are to be entered into the study provided they conform to the following criteria.

9.2. Inclusion Criteria

Subjects must meet *all* of the following inclusion criteria to be eligible for participation in this study.

1. Subjects must be considered by the investigator to be in general good health and between 18 and 65 years of age, inclusive.
2. Subjects must have a history of smoking at least 10 cigarettes per day for at least 1 year, with less than 3 months of abstinence during the past year, and a continuous smoking history for at least 3 months prior to study entry.
3. Subjects must indicate that they plan to quit smoking within the next 3 months.
4. Subjects must have a CO measurement of $\geq$ 10 ppm at baseline.
5. Subjects must be willing and able to return for scheduled follow-up examinations for a total of 5 months.
6. Female subjects must have been postmenopausal for the previous 6 months or be surgically sterile, or must have a negative pregnancy test and use one of the following acceptable methods of birth control for the duration of the study and for at least 3 months prior to screening:
    a. abstinence, meaning a total lack of sexual activity
    b. oral contraceptives (“the pill”),
    c. contraceptive injections,
    d. intrauterine device,
    e. double-barrier method (diaphragm or condom plus spermicidal cream)

f. contraceptive patch, hormonal implant, hormonal vaginal ring, or

g. male partner sterilization at least 3 months prior to screening.

7. Subjects must sign and be given a copy of the written Informed Consent form.

8. Subjects must be able to read, understand, and complete the questionnaires independently.

9.3. Exclusion Criteria

Subjects who meet *any* of the following exclusion criteria are not to be enrolled in this study.

1. Subjects who have a household member who is already enrolled in this trial.

2. Subjects who have attempted to quit smoking using one or more FDA-approved smoking cessation products (eg, NRT including nicotine gum, nicotine lozenges, nicotine patches, nicotine nasal spray, or nicotine inhaler; Zyban [bupropion]; or Chantix [varenicline]) for longer than 1 week in the past 3 months.

3. Subjects who have been enrolled in another structured smoking cessation program (e.g., behavioral modification, hypnosis, acupuncture, or other alternative therapies) in the last 30 days.

4. Subjects who have been in another smoking cessation trial in the past 6 months.

5. Subjects who regularly use (greater than once per month) tobacco-based products other than cigarettes (eg, chew, snuff, snus, shisha, etc.).

6. Subjects with a history of coronary artery disease, myocardial infarction, stroke, chronic obstructive pulmonary disease, or other significant pulmonary diseases. Controlled hypertensive subjects and mild asthmatics whose disease is controlled only with an as-needed inhaled beta agonist are acceptable.

7. Subjects with a history of cancer within the past 60 months other than basal cell carcinoma.

8. Subjects with a history of thromboembolic disease or taking warfin or any other anticoagulant, or anti-platelet drug such as clopidogrel (Plavix®) or pentoxifylline (Trental®). Low dose aspirin therapy is acceptable.

9. Subjects with screening laboratory abnormalities that are considered by the investigator to be clinically significant.

10. Subjects with a body mass index (BMI) >35.

11. Subjects with poorly controlled diabetes or insulin-dependent diabetes.
12. Subjects with other known serious pathophysiology or topical or systemic disorders of any kind that would confound the results of the study.
13. Subjects with screening ECG abnormalities that are considered by the investigator to be clinically significant.
14. Subjects who have used any illegal drug in the past 3 months.
15. Subjects who have a positive urine drug screening result.
16. Subjects who are pregnant or lactating, or who plan to become pregnant during the course of the study.
17. Subjects participating in any other clinical trial of an investigational drug or device during the time of this clinical investigation or within 30 days prior to Screening visit.
18. Subjects taking anti-depressants, anti-psychotics, clonidine, or any of the classes of drugs listed in the RapidCHECK® 12 Panel in the last 60 days (see Section 12.1.1).
19. Subjects with systolic blood pressure over 140 mmHg-and/or diastolic blood pressure over 90 mmHg.
20. Subjects consuming an average of 3 or more drinks of alcohol per day.
21. Subjects consuming greater than an average of 2 packs of cigarettes per day.

9.4. Removal of Subjects from the Study

A subject will be considered to have completed the study when he or she completes the Week 10 visit. In the absence of a medical contraindication or significant protocol violation, every effort will be made by the investigator to keep the subject in the study through Week 18 (Visit 9). If a subject is discontinued prematurely at any time after entering the study, the investigator will make every effort to see the subject and complete the Week 10 assessments as shown in the Schedule of Assessments (Appendix 1).

All subjects have the right to withdraw at any point during treatment without prejudice. The investigator can discontinue a subject at any time if medically necessary.

A termination case report form (CRF) page should be completed for every subject who received study medication, whether or not the subject completed the study. The reason for any early discontinuation should be indicated on this form. The primary reason for a subject withdrawing prematurely should be selected from the following standard categories of early termination:

- *Adverse Event*: Events occurred that in the medical judgment of the investigator for the best interest of the subject are grounds for discontinuation. This includes serious and nonserious AEs regardless of relationship to study materials.
- *Death*: The subject died.
- *Withdrawal of Consent*: The subject desired to withdraw from further participation in the study in the absence of an investigator-determined medical need to withdraw. If the subject gave a reason for withdrawing, it should be recorded in the CRF.
- *Protocol Violation*: The subject's findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (e.g., noncompliance, failure to return for defined number of visits). The violation necessitated premature termination from the study.
- *Lost to Follow-Up*: The subject stopped coming for visits, and study personnel were unable to contact the subject.
- *Other*: The subject was terminated for a reason other than those listed above, such as theft or loss of study materials or termination of study by sponsor.

10. TREATMENTS

10.1. Description of Study Treatments

10.1.1. X-22 Very Low Nicotine Cigarette

The X-22 Smoking Cessation Product is a tobacco-based (botanical) medical product consisting of VLN cigarettes. X-22 cigarettes, like conventional cigarettes, consist of filler, filter, and wrapper components. X-22 cigarettes contain menthol, (b) (4) The menthol is included in part to compensate for the reduced sensory impact of the cigarettes in the mouth and throat due to the very low nicotine content.

The filler in X-22 cigarettes, like that in conventional cigarettes, is comprised primarily of cured tobacco leaf and does not contain non-tobacco plant material (other than a relatively small amount present in some of the additives and flavorings included in the filler). The filler of X-22 cigarettes contains a proprietary VLN tobacco variety, Vector 21-41, which has very low levels of nicotine due to genetic modification of the nicotine biosynthesis pathway in the plant. X-22 cigarettes have a tobacco content and tar yield comparable to typical commercial cigarettes, but have a nicotine content of less than 1 mg/g and a machine smoking yield of ≤0.05 mg of nicotine per cigarette, a reduction of more than 95% compared to typical commercial cigarettes.

All processing of tobacco for X-22 is carried out by processors of conventional tobacco. All manufacturing of cigarette components and cigarettes is carried out by manufacturers of conventional cigarette components and cigarettes.

10.1.2. Control Cigarettes

The Active Control cigarette is identical to the X-22 cigarette except for the filler, which has a nicotine content similar to that of a conventional “light” cigarette with a machine smoking yield of approximately 0.7 mg nicotine per cigarette. The Active Control cigarette, like the X-22 cigarette, contains menthol. The objective of the Active Control is to deliver smoke with a typical nicotine concentration from a product with the same appearance as X-22.

10.2. Randomization and Blinding

Subject will be randomized in a 1:1 ratio of X-22 to active control stratified by gender.

All potential subjects will be assigned a screening number, and those failing screening will be replaced until 216 eligible subjects have been randomized. Those subjects who are screened and considered to be eligible for the study will be assigned a Subject Identification number upon randomization. Prematurely discontinued subjects will not be replaced.

Unblinding

This is a double-blind study. The Investigator, sub-Investigators, study site staff, Sponsor personnel or designees, and subjects will be blinded as to the nature of the treatment (X-22 cigarettes or active control cigarettes) assignment. The study randomization schedule will be kept in a secure location by the study statistician. The contact information for the study statistician is included in the X-22-201 Safety Monitoring Plan.

The X-22-201 Medical Monitor will have access to the randomization assignment of individual subjects from the study statistician in case an SAE is reported to 22nd Century and is considered at least possibly related to study medication, so that the blind may be broken if necessary for alert reporting requirements.

Only in the case of an emergency, when knowledge of the investigational product is essential for the clinical management or welfare of the subject, may the Investigator unblind a subject's treatment assignment. If possible, the Investigator should consult with the Medical Monitor prior to contacting the study statistician regarding a subject's treatment assignment. However, if the medical monitor is not available, the Investigator may contact the study statistician directly to unblind a subject's treatment assignment.

If the blind is broken for any reason, the Investigator must notify the Sponsor immediately of the unblinding incident without revealing the subject's study treatment assignment. If the blind was broken to treat an SAE that the Investigator considered at least possibly related to study treatment, then the Sponsor will unblind itself as in accordance with alert reporting requirement described in Section 12.3.4.6. The Investigator will record the date and reason for breaking the treatment blind for any subject in the CRF.

10.3. Treatment Dispensing and Monitoring

Cartons of study cigarettes will be dispensed at Visits 2, 3, and 4. Subjects will maintain a diary recording all tobacco and NRT products used during the study. The diary and all unused study cigarettes must be returned to the site at each study visit. Returned unused cigarettes will be counted and recorded.

10.4. Packaging and Labeling

All study products will bear the statements “Caution: New Drug – Limited by United States law to investigational use”. Additionally, cigarette packs will communicate the following Surgeon General's warning:

"SURGEON GENERAL'S WARNING: Cigarette Smoke Contains Carbon Monoxide."

The trial cigarettes will be packaged in a manner similar to conventional cigarettes, 20 cigarettes per pack and 10 packs per carton. The packs are blank except for the investigational material cautions, trial protocol identification, and the Surgeon General’s Warning. X-22 cigarettes and the Active Control cigarettes will be packaged in identical packs, such that there will be no distinctive visible markings.

The packs are wrapped in cellophane and packaged 10 packs to a carton. The carton will contain the investigational material cautions, protocol number, and the Surgeon General’s Warning. Cartons of X-22 cigarettes and Active Control cigarettes are distinguished only by the coded Subject ID number on the label applied when the trial materials are packed into kits for distribution to study sites.

10.5. Storage and Handling

Access to the investigational product should be strictly limited to the investigators and their designees. Neither the investigators nor any designee may provide the investigational product to any subject not enrolled in this protocol.

The investigational product should be stored in a secured area at controlled room temperature.

10.6. Dosage and Administration of X-22 Smoking Cessation Product and Active Control Cigarette

Investigational product will be dispensed at study Visits 2, 3, and 4. At Visit 2, the number of cigarettes dispensed will be approximately equal to the number of cigarettes the subject has reported smoking in a 2-week period plus an overage of 20%. The number of cigarettes dispensed may be adjusted at Visits 3 and 4 as necessary up to a maximum of 11 cartons for the 6-week Treatment Period. Subjects will be instructed to telephone the site to request additional study cigarettes during the treatment period, if necessary.

Subjects may consume the study cigarettes as desired, and must record all cigarette, other tobacco, and NRT use in a diary provided by the Sponsor. Unused cigarettes must be returned to the site at each subsequent study visit.

10.7. Prior and Concomitant Medications

All medications taken by the subject within 30 days of study entry must be recorded at the Screening visit (Visit 1) on the prior medications CRF page. All medications taken by a subject at any time during the study must be recorded on the concomitant medications CRF page, including the route, indication, and dates of use.

11. STUDY PROCEDURES

The study procedures to be conducted for each subject enrolled in the study are presented in Appendix 1 and detailed in the text that follows. Additional information on the study procedures is provided in the Study Procedures Manual. All study procedures are to be performed by the investigator or other qualified study site personnel.

Any deviation from protocol procedures should be noted in the CRF and the Sponsor or their designee should be notified.

11.1. Study Assessments by Visit

11.1.1. Visit 1: Screening (Day -7 ± 2 Days)

Subjects will be screened within 7 days prior to randomization to determine eligibility for participation in the study. The following will be performed and documented at Screening:

- Signed written informed consent will be obtained.
- Screening for inclusion and exclusion criteria will be completed.
- Subjects will be asked if they plan to quit smoking within the next 3 months.
- A urine sample for drug screening will be obtained.
- A blood sample will be drawn for clinical laboratory tests.
- Duplicate copies of a single ECG tracing will be obtained.
- Demographic information will be collected.
- Medical history will be taken.
- Smoking history (including number of cigarettes smoked per day, and documentation of current usual brand) will be taken.
- Record concomitant medications taken within the previous 30 days.
- Physical examination will be performed.
- Urine pregnancy testing will be performed for women of childbearing potential.
- Alcohol intake will be assessed.
- Blood pressure, heart rate, temperature, and respiratory rate will be measured.

- Height will be measured.
- Weight will be measured.
- Exhaled CO concentrations will be measured.
- Saliva will be analyzed for cotinine.
- Sensory Questionnaire will be completed (for usual brand) by the subject.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Fagerström Test of Nicotine Dependence will be completed by the subject.
- Diary card and instructions for completing and returning it will be given.
- Subjects will be instructed to smoke their usual brand cigarettes *ad libitum* until Visit 2.
- Subjects meeting all of the inclusion criteria and none of the exclusion criteria will return to the study site in 7 days (± 2 days) for Visit 2.

11.1.2. Visit 2: Baseline and Randomization (Day 0)

The following procedures and observations will be performed:

- Inclusion and exclusion criteria will be reviewed.
- Randomization will be completed.
- Concomitant medications taken since the last visit will be recorded.
- Blood pressure and heart rate will be measured.
- Weight will be measured.
- Diary cards will be returned to the site to document cigarette, other tobacco, and NRT usage. Entries will be reviewed with subject, feedback provided, and a new diary card dispensed.
- Exhaled CO concentrations will be measured.
- Saliva will be analyzed for cotinine.
- Sensory Questionnaire will be completed (for usual brand) by the subject.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Fagerström Test of Nicotine Dependence will be completed by the subject.

- Adverse events will be reviewed and recorded.
- Subjects will be scheduled to return to the site in 2 weeks (± 2 Days) for Visit 3.
- First 2 weeks of randomized treatment will be dispensed and the subject will be instructed to telephone the site to request additional study cigarettes prior to the next visit, if necessary.
- Subjects will be instructed to bring their unused study cigarettes and diary card to the site at the next visit.

11.1.3. Week 1 Telephone Calls

At Week 1 (± 2 days), a representative of the study site will telephone the subject to ensure that the subject has an adequate supply of investigational product and to obtain information regarding AEs.

On the day before Visit 3, a representative of the study site will telephone the subject to remind them of their Visit 3 appointment.

11.1.4. Visit 3: Treatment Period (Week 2 ± 2 Days)

At this visit, the following procedures and observations will be performed:

- Concomitant medications taken since the last visit will be recorded.
- Blood pressure and heart rate will be measured.
- Weight will be measured.
- Diary cards will be returned to the site to document cigarette, other tobacco, and NRT usage, and a new diary card will be dispensed.
- Exhaled CO concentrations will be measured.
- Saliva will be analyzed for cotinine.
- Sensory Questionnaire will be completed by the subject.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Fagerström Test of Nicotine Dependence will be completed by the subject.
- Unused study cigarettes will be returned and the number of returned cigarettes will be recorded.
- Adverse events will be reviewed and recorded.

- Subjects will be scheduled to return to the site in 2 weeks (± 2 days) for Visit 4.
- The second 2 weeks of assigned randomized treatment will be dispensed and the subject will be instructed to telephone the site to request additional study cigarettes prior to the next visit, if necessary.
- Subjects will be reminded to bring their unused study cigarettes and diary to the site at the next Visit.

11.1.5. Week 3: Telephone Calls

At Week 3 (± 2 days), a representative of the study site will telephone the subject to ensure that the subject has an adequate supply of investigational product and to obtain information regarding AEs.

On the day before Visit 4, a representative of the study site will telephone the subject to remind them of their Visit 4 appointment.

11.1.6. Visit 4: Treatment Period (Week 4 ± 2 days)

At this study visit, the following procedures and observations will be performed:

- Concomitant medications taken since the last visit will be recorded.
- Blood pressure and heart rate will be measured.
- Weight will be measured.
- Exhaled CO concentrations will be measured.
- Saliva will be analyzed for cotinine.
- Sensory Questionnaire will be completed by the subject.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Fagerström Test of Nicotine Dependence will be completed by the subject.
- Approximately 15-minute counseling session appropriate to the phase of the study will be provided and supporting printed materials will be dispensed.
- Diary card will be returned to the site to document cigarette, other tobacco, and NRT usage, and new diary card will be dispensed.
- Unused study cigarettes will be returned and the number of returned cigarettes will be recorded.

- Adverse events will be reviewed and recorded.
- Subjects will be scheduled to return to the site in 2 weeks (± 2 days) for Visit 5.
- Two weeks of assigned randomized treatment will be dispensed and the subject will be instructed to telephone the site to request additional study cigarettes prior to the next visit, if necessary.
- Subjects will be reminded to bring their unused study cigarettes and diary to the site at the next visit.

11.1.7. Week 5 Telephone Calls

At Week 5 (± 2 days), a representative of the study site will telephone the subject to ensure that the subject has an adequate supply of investigational product and to obtain information regarding AEs.

On the day before Visit 5, a representative of the study site will telephone the subject to remind them of their Visit 5 appointment.

11.1.8. Visit 5: Quit Date (Week 6 ± 2 days)

At this study visit, the following procedures and observations will be performed:

- Concomitant medications taken since the last visit will be recorded.
- A blood sample will be drawn for clinical laboratory tests.
- Blood pressure and heart rate will be measured.
- Weight will be measured.
- Exhaled CO concentrations will be measured.
- Saliva will be analyzed for cotinine.
- Sensory Questionnaire will be completed by the subject.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Fagerström Test of Nicotine Dependence will be completed by the subject.
- Approximately 15-minute counseling session appropriate to the phase of the study will be provided and supporting printed materials will be dispensed.
- Diary cards will be returned to the site to document cigarette, other tobacco, and NRT usage, and a new diary card will be dispensed.

- Unused study cigarettes will be returned and the number of returned cigarettes will be recorded.
- Adverse events will be reviewed and recorded.
- Subjects will be scheduled to return to the site in 2 weeks (± 2 days) for Visit 6.

11.1.9. Week 7 Telephone Calls

At Week 7 (± 2 days), a representative of the study site will telephone the subject and obtain information regarding AEs. Subjects will be asked if they have smoked in the last 7 days.

On the day before Visit 6, a representative of the study site will telephone the subject to remind them of their Visit 6 appointment.

11.1.10. Visit 6: 2-Week Abstinence Assessment (8 Weeks ± 2 Days)

This visit must occur within 12 to 16 days after the quit date. At this visit, the following procedures and observations will be performed:

- Concomitant medications taken since the last visit will be recorded.
- Blood pressure and heart rate will be measured.
- Weight will be measured.
- Exhaled CO concentrations will be measured.
- Diary cards will be returned to the site to document cigarette, other tobacco, and NRT usage, and a new diary card will be dispensed.
- Saliva will be analyzed for cotinine.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Approximately 15-minute counseling session appropriate to the phase of the study will be provided and supporting printed materials will be dispensed.
- All subjects will be asked if they have smoked since the last visit and if they have smoked within the last 7 days.
- Adverse events will be reviewed and recorded.
- Subjects will be scheduled to return to the site in 2 weeks (± 2 days) for Visit 7.

11.1.11. Week 9 Telephone Calls

At Week 9 (± 2 days), a representative of the study site will telephone the subject and obtain information regarding AEs. Subjects will be asked if they have smoked in the last 7 days.

On the day before Visit 7, a representative of the study site will telephone the subject to remind them of their Visit 7 appointment.

11.1.12. Visit 7: 4-Week Abstinence Assessment (Week 10 + up to 4 days)/ Early Termination

This visit must occur within 28 to 32 days after the quit date. The following procedures and observations will be performed at this visit and for any subject who terminates the study prematurely:

- Concomitant medications taken since the last study visit will be recorded.
- Physical examination will be performed.
- Urine pregnancy testing will be performed for women of childbearing potential.
- Blood pressure, heart rate, temperature, and respiratory rate will be measured.
- Weight will be measured.
- Exhaled CO concentrations will be measured.
- Diary cards will be returned to the site to document cigarette, other tobacco, and NRT usage, and a new diary card will be dispensed.
- Saliva will be analyzed for cotinine.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Subjects will be asked to guess which treatment group they were assigned to.
- All subjects will be asked if they have smoked since the last visit and if they have smoked within the last 7 days.
- Adverse events will be reviewed and recorded.
- Subjects will be notified that they will receive a follow-up phone call in approximately 1 month to determine their smoking status.
- Note: subjects who terminate the study prematurely prior to Visit 5 should also have a blood sample drawn for clinical laboratory tests at the early termination visit.

Subjects who complete this study visit will be considered to have completed the study.

11.1.13. Pre-week 14 Telephone Calls (Week 13 + up to 3 days)

This telephone call must take place 4 to 7 days before Week 14. Subjects will be questioned regarding their smoking status by telephone. Subjects who indicate that they have smoked within the past 7 days will be questioned about AEs and will be notified that they will receive another follow-up phone call in approximately 1 month to determine their smoking status. Subjects who indicate that they have not smoked within the past 7 days will be asked to come to the site within 1 week.

11.1.14. Visit 8: 2-Month Follow-up (Week 14 ± 2 days)

Subjects who indicated during their Week 14 telephone call that they had not smoked within the past 7 days will come to the site for the following procedures and observations:

- Concomitant medications taken since the last visit will be recorded.
- Blood pressure and heart rate will be measured.
- Weight will be measured.
- Exhaled CO concentrations will be measured.
- Diary cards will be returned to the site to document cigarette, other tobacco, and NRT usage, and a new diary card will be dispensed.
- Saliva will be analyzed for cotinine.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- Adverse events will be reviewed and recorded.
- All subjects will be asked if they have smoked since the last visit and if they have smoked within the last 7 days.
- Subjects will be scheduled to return to the site in 4 weeks (± 2 days) for Visit 9.

11.1.15. Week 18 Telephone Call

On the day before Visit 9, a representative of the study site will telephone the subject to remind them of their Visit 9 appointment.

11.1.16. Visit 9: 4-Month Follow-up (Week 18 ± 2 days)

All subjects will return to the site for the final follow-up visit and the following procedures and observations:

- Concomitant medications taken since the last visit will be recorded.
- Blood pressure and heart rate will be measured.
- Weight will be measured.
- Exhaled CO concentrations will be measured.
- Diary cards will be returned to the site to document cigarette, other tobacco, and NRT usage.
- Saliva will be analyzed for cotinine.
- Minnesota Nicotine Withdrawal Scale will be completed by the subject.
- All subjects will be asked if they have smoked since the last visit and if they have smoked within the last 7 days.
- Adverse events will be reviewed and recorded.
- Approximately 15-minute counseling session appropriate to the phase of the study will be provided, including information regarding approved smoking cessation pharmacotherapies to those subjects who have not yet quit smoking, and supporting printed materials will be dispensed.

12. DESCRIPTION OF ASSESSMENTS

12.1. Screening Assessments

12.1.1. Urine Drug Screen

A urine sample will be obtained to perform the drug screen at Visit 1. The RapidCHECK® 12 Panel will be used. This test is able to detect the analytes listed in Table 12-1, below.

Table 12-1 Analytes Detected in Urine Drug Screen

Symbol	Analyte	Cut-off Level
THC	Marijuana	50 ng/mL
COC	Cocaine	300 ng/mL
PCP	Phencyclidine	25 ng/mL
MOR	Opiates	2000 ng/mL
MET	Methamphetamine	1000 ng/ml
MTD	Methadone	300 ng/ml
AMP	Amphetamine	1000 ng/mL
BAR	Barbiturates	300 ng/mL
BZO	Benzodiazepines	300 ng/mL
OXY	Oxycodone	100 ng/mL
MDMA	MDMA Ecstasy	500 ng/mL
PPX	Propoxyphene	300 ng/mL

12.1.2. Pregnancy Tests

All female subjects, excluding those who have been post menopausal for 1 year or who are surgically sterile, will undergo a urine pregnancy test at Visits 1 and 7. Test results will be evaluated by the site and must be negative at Screening in order for the subject to be eligible for study participation.

12.1.3. Electrocardiogram

Duplicate copies of a single 12-lead ECG tracing will be obtained at Screening and evaluated at the investigational site to determine the subject's eligibility for the study. Additional 12-lead ECGs may be obtained at the investigator's discretion.

12.1.4. Medical History

Relevant medical history over the previous 5 years will be recorded at Screening.

12.2. Efficacy Assessments

The primary efficacy measurement is the number of cigarettes smoked, as assessed by subject diary and confirmed by exhaled CO, with the primary endpoint being the number of subjects achieving 4-weeks of abstinence from smoking as assessed at Visits 6 and 7 (Weeks 8 and 10).

Secondary efficacy measurements include withdrawal symptoms as measured by the Minnesota Nicotine Withdrawal Scale. The degree of dependence on smoking will be measured at Screening and during the Treatment Phase using the Fagerström Test of Nicotine Dependence. The sensory questionnaire will be administered to obtain detailed information about the sensory properties of the X-22 cigarettes.

An approximately 15-minute counseling session appropriate to the phase of the study will be provided at Visits 4, 5, 6, and 9 to each subject and supporting printed materials will be dispensed.

All efficacy measurements will be taken at the times indicated in the Schedule of Assessments (see Appendix 1and Section 11.1).

12.2.1. Counseling Session and Written Materials

An approximately 15-minute smoking cessation counseling session will be provided to each subject at Visits 4, 5, 6, and 9. Site personnel will be trained by Quit Smart® Stop Smoking Resources, Inc. to ensure consistent counseling across study sites. The information presented at each session will be adjusted to be consistent with the current phase of the study. Supporting printed materials created by Quit Smart® specifically for this study will be provided to all study subjects during the counseling sessions. Details regarding the printed materials and counseling sessions are provided in the Study Procedures Manual. The Visit 9 counseling session will include information regarding the use of approved smoking cessation pharmacotherapies.

12.2.2. Smoking Diary Cards

Each subject will be given a new smoking diary card at Visits 1 to 8. Subjects will be instructed to complete the card each day and to record the number of cigarettes smoked, both study cigarettes and any other cigarettes, as well as any other tobacco products or nicotine replacement products used. The subject should bring their completed diary cards to each

subsequent study visit (Visits 2 to 9). At Visit 2, study personnel should review the subject's completed diary card with the subject to ensure proper completion and to provide any necessary feedback. Diary cards will be retained with the source documentation for that subject.

12.2.3. Saliva Cotinine Concentration Measurements

Because approximately 80 percent of nicotine is transformed to cotinine in humans, saliva cotinine levels are used as one of the measures of nicotine use. NicAlert™ (Nymox Pharmaceutical Corporation, Hasbrouk Heights, NJ) will be used to measure cotinine levels as described in the Study Procedures Manual. To maintain the study blind, care will be taken at the site to ensure that analysis of each sample and recording of results are conducted by a person not involved in other study assessments in an area out of view of other study personnel.

12.2.4. Carbon Monoxide Concentration Measurements

Subject's exhaled CO concentrations will be measured using a portable CO monitor (Vitalograph, Inc., Lenexa, KS) as described in the Study Procedures Manual.

12.2.5. Minnesota Nicotine Withdrawal Scale

The Minnesota Nicotine Withdrawal Scale is provided in The Study Procedures Manual. This is a widely-used questionnaire that will be administered to assess craving, negative affect, arousal, somatic symptoms, appetite, and habit. The original version of this scale was published in 1986.[29] During this study, the current version of the self-report scale[30] will be used at the times indicated in Appendix 1.

12.2.6. Sensory Questionnaire

The Sensory Questionnaire is provided in The Study Procedures Manual. This questionnaire will be administered to obtain detailed information about the sensory properties of the study cigarettes.

12.2.7. Fagerström Test of Nicotine Dependence

The Fagerström Test of Nicotine Dependence is provided in The Study Procedures Manual. This questionnaire will be administered to measure the degree of physical dependence on tobacco smoking. This scale was adapted from the Fagerström Tolerance Questionnaire and

was first published in 1991.[31,32] It is commonly used in studies of smoking behavior and cessation.

12.2.8. Subject Guess Regarding Treatment Group

At Visit 7, subjects will be asked to guess which treatment group they were enrolled in during the study. Subjects will indicate, by circling a value on an analog scale, which treatment they think they received.

12.3. Safety Assessments

All AEs spontaneously reported, elicited, or observed by the investigators will be recorded. Heart rate, blood pressure, and body weight will be measured at each study visit. Physical examinations will be conducted at Screening and Visit 7. Clinical laboratory tests will be performed at Screening and Visit 5.

All safety measurements will be taken at the times indicated in the Schedule of Assessments (see Section 11.1 and Appendix 1).

12.3.1. Physical Examination

A limited system-by-system physical exam should be conducted and documented in the source medical chart at the times indicated in Section 11.1 and Appendix 1. The following will be evaluated:

- Height (in centimeters), weight (in kilograms), general appearance, and vital signs (including heart rate, blood pressure, respiratory rate, and temperature [degrees centigrade]);
- Ear, nose, and throat;
- Eye examination including fundoscopy;
- Heart;
- Chest;
- Abdomen;
- Extremities;
- Musculoskeletal;
- Skin;

- Nervous system; and
- Other - any additional assessments necessary to establish that the subject is considered 'healthy' as required by inclusion criteria, to establish baseline status, or to evaluate symptoms or AEs.

Body weight will also be recorded in kilograms at each subsequent study visit.

12.3.2. Vital Signs

Heart rate and blood pressure will be measured and recorded at each study visit (respiratory rate and temperature will only be recorded in conjunction with physical examinations at Visits 1 and 7).

12.3.3. Clinical Laboratory Tests

The laboratory tests listed in Table 12-2 will be performed at Screening and Visit 5. The results of screening laboratory tests must be reviewed by the investigator prior to randomization. Additional blood samples may be collected for further evaluation of safety as warranted by the investigator's judgment. It is recommended that subjects fast for a minimum of 8 hours prior to all blood draws for hematology and serum chemistry assessments.

Table 12-2 Clinical Laboratory Tests

Clinical Chemistry	Hematology
Blood urea nitrogen	White blood count with differential
Creatinine	Red blood cell (RBC) count
Aspartate transaminase (AST or SGOT)	Hematocrit
Alanine transaminase (ALT or SGOT)	Hemoglobin
Lactate dehydrogenase	Mean corpuscular volume (MCV)
Total bilirubin	Mean corpuscular hemoglobin concentration (MCHC)
Chloride	Mean platelet volume (MPV)
Carbon dioxide	Platelets
Glucose	
Sodium	
Potassium	
Gamma glutamyl transferase (GGT)	

12.3.4. Adverse Events

The investigator is responsible for the detection and documentation of events that meet the definition of an adverse event (AE), or serious adverse event (SAE). Definitions and terminology relevant to reporting of AEs are described below.

12.3.4.1. Operating Definitions for Assessing Safety

Safety data collection for this study begins at the time of the subject's informed consent according to the operating definitions defined in this section of the protocol. The investigator is responsible for the detection and documentation of events that meet the definition of an unanticipated problem, AE, suspected adverse reaction, adverse reaction, or SAE. Definitions and terminology relevant to IND Safety Reports as referenced in the U.S. Code of Federal Regulations are addressed below.

Definition of an Adverse Event:

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

An AE **does** include the following:

- Exacerbation of a pre-existing illness.
- Increase in frequency of a pre-existing episodic event or condition.
- Condition detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Symptom associated with disease not previously reported/identified for the subject will be as an AE. NOTE: If the pre-existing illness is the disease under study, then "exacerbation" refers to an unexpected worsening from the condition at baseline.

An AE **does not** include the following:

- Medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE if it meets the definition of an AE.
- Pre-existing diseases or conditions that worsen during the course of the study. The disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the subject's condition.

- Symptom(s) associated with the disease under study not previously reported by the subject will be recorded as an AE, only if it is more severe than expected, or unless the symptom(s) meet the criteria for an SAE.

Definition of a Suspected Adverse Reaction:

Suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

The following examples are the types of evidence that would suggest a causal relationship between the drug and the AE and would justify the consideration of an event as a suspected adverse reaction:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture),
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Suspected adverse reactions are the subset of all AEs for which there is a reasonable possibility that the drug caused the event. Inherent in this definition, and in the requirement to report them, is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the drug actually caused the AE. FDA considers the definition of suspected adverse reaction and the application of the reasonable possibility causality standard to be consistent with the concepts and discussion about causality in the International Conference on Harmonization (ICH) E2A guidance.

Definition of Unexpected Adverse Event or Unexpected Suspected Adverse Reaction:

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the

investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

This definition relies entirely on a listing of the AEs or suspected adverse reactions in the investigator brochure (or elsewhere in the general investigational plan if an investigator brochure is not required or available) as the basis for determining if newly acquired information generated from clinical trials or reported from other sources is unexpected. The suspected adverse reactions listed in the investigator's brochure (i.e., "expected") are those observed with the investigational drug and for which a causal relationship between the event and the drug is suspected or confirmed. Thus, AEs that would be anticipated to occur as part of the disease process are considered unexpected for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure. In addition, some AEs are listed in the investigator brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered unexpected until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class, the first case of angioedema observed with the drug under investigation should be considered unexpected for reporting purposes.

Definition of a Serious Adverse Event or Serious Suspected Adverse Reaction:

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death.
- A life-threatening AE: An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization, the event is an SAE. "Inpatient" hospitalization means the subject has been formally admitted to a hospital for medical reasons. This may or may not be overnight. It does not include presentation at a casualty or emergency room.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- A congenital anomaly/birth defect,
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependence or drug abuse.

This definition permits either the sponsor or the investigator to decide if an event is serious. Because SAEs are critically important for the identification of significant safety problems, FDA believes taking into account both the investigator's and the sponsor's assessment is important. For example, the investigator's perspective may be informed by having actually observed the event, and the sponsor is likely to have broader knowledge of the drug and its effects to inform its evaluation of the significance of the event. If either the sponsor or investigator believes that the event is serious, the event must be considered serious and evaluated by the sponsor for expedited reporting.

12.3.4.2. Clinical Laboratory Abnormalities and Other Abnormal Assessments:

Abnormal laboratory findings (eg, clinical chemistry, hematology) or other abnormal assessments (eg, abnormal findings during examinations) that are judged by the investigator as clinically significant must be recorded as AEs, suspected adverse reactions, or SAEs if they

meet the definition of an AE, suspected adverse reaction, or an SAE as defined above. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected after investigational product administration or that are present at Baseline and worsen following the start of the investigational product are included as AEs, suspected adverse reactions, and SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease or condition being studied (unless judged by the investigator as more severe than expected for the subject's condition), or that are present or detected at the start of the study that do not worsen, are not included as AEs, suspected adverse reactions, or SAEs.

The investigator should exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

12.3.4.3. Adverse Event Assessment

Each AE or suspected adverse reaction must be described as follows: the date of onset, date of resolution, severity, frequency of the event (single episode, intermittent, continuous), action taken with study treatment (no action taken, treatment held, treatment discontinued), outcome, and seriousness criteria must be recorded. Each AE or suspected adverse reaction must be recorded separately.

Severity will be assessed using the definitions in Table 12-3:

Table 12-3 Severity of Adverse Events

Severity	Definition
Mild	Aware of sign or symptom, but easily tolerated
Moderate	Discomfort enough to cause interference with usual activity
Severe	Incapacitating with inability to work or do usual activity

Relationship of the AE to the investigational product will be assessed using the definitions in Table 12-4.

Table 12-4 Relationship of Adverse Events to the Investigational Product

Relationship	Definition
Not related	Evidence exists that the Adverse Event definitely has a cause other than the investigational product (e.g. pre-existing condition or underlying disease, intercurrent illness, or concomitant medication) and does not meet any other criteria listed below.
Possibly related	A temporal relationship exists between the event onset and administration of investigational product. Although the event may appear unlikely to be related to the investigational product, it cannot be ruled out with certainty; and / or the event cannot be readily explained by the subject's clinical state or concomitant therapies. Events that are possibly or probably related to the administration of the investigational product are defined as Suspected Adverse Reactions.
Probably related	A temporal relationship exists between the event onset and administration of investigational product, and appears with some degree of certainty to be related based on known therapeutic and pharmacologic actions of the investigational product. It cannot be readily explained by the subject's clinical state or concomitant therapies. Events that are possibly or probably related to the administration of the investigational product are defined as Suspected Adverse Reactions.
Definitely related	Strong evidence exists that the investigational product caused the AE. There is a temporal relationship between the event onset and administration of the investigational product. There is strong therapeutic and pharmacologic evidence that the event was caused by the investigational product. The subject's clinical state and concomitant therapies have been ruled out as a cause. Adverse Reactions are a subset of all Suspected Adverse Reactions for which there is reason to conclude that the drug caused the event. Events that are definitely related to administration of the investigational product are defined as Adverse Reactions.

Outcome will be assessed using the following categories: recovered/resolved, not recovered/not resolved, recovered/resolved with sequelae, fatal, or unknown.

12.3.4.4. Method, Frequency, and Time Period for Detecting Adverse Events

At each visit, after the subject has had an opportunity to spontaneously mention any problems, the investigator should inquire about AEs by asking non-leading questions such as the following:

- “How are you feeling?”
- “Have you had any (other) medical problems since your last visit/assessment?”

- "Have you taken any new medicines, other than those given to you in this study, since your last visit/assessment?

In the case that a subject was seen by a different health care professional from the investigator (eg, at a different institution) in relation to an AE or adverse reaction, every effort should be made by the investigator to contact the treating physician in a timely manner in order to obtain all necessary information and report the event appropriately.

Post-Study SAEs:

Investigators are not obligated to actively seek SAE information in former study participants, but investigators are encouraged to notify the sponsor or designee of any SAEs occurring at any time after a subject has discontinued or terminated study participation that they judge may be reasonably be related to study treatment or study participation.

12.3.4.5. Adverse Event Documentation

All subjects who have been exposed to the investigational product will be evaluated for AEs. All AEs that occur after informed consent is provided will be recorded. AEs that occur before the time of treatment with the investigational product will be considered not to be treatment emergent AEs (TEAEs) and all AEs that occur after the time of treatment with the investigational product will be considered TEAEs. Each AE or suspected adverse reaction must be recorded separately.

Any AE occurring during the study must be documented in the subject's medical records and as an AE in the CRF. Any SAE occurring during the study must be documented in the subjects' medical records and as an SAE in the CRF.

A separate set of SAE pages should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporarily and/or clinically related, they may be reported on the same SAE page.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs or symptoms.

For clinically significant abnormal laboratory findings or other abnormal assessment that meets the definition of an AE or SAE, a diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, should be entered in the CRF as an AE or SAE. If no diagnosis is

known and clinical signs and symptoms are not present, the abnormal finding should be recorded.

The investigator will take all appropriate and necessary therapeutic measures required for resolution of the AE. Any medication necessary for the treatment of an AE must be recorded on the concomitant medication case report form.

The SAE pages of the CRF should be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the sponsor. It is very important that the investigator provide his/her assessment of causality to investigational product at the time of the initial SAE report.

12.3.4.6. Prompt Reporting of Serious Adverse Events

Serious adverse events must be reported promptly to the sponsor or designee once the investigator determines that the event meets the protocol definition of an SAE or serious suspected adverse reaction. Prompt reporting of an SAE/serious suspected adverse reaction requires:

- Completion and transmission of SAE CRF pages to sponsor or designee within 24 hours of the investigator's knowledge of the event. The SAE pages of the CRF should be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the sponsor or designee. It is important that the investigator provide his/her assessment of causality to the investigational product at the time of initial reporting of the SAE.
- Prompt reporting of additional information for previously reported SAEs should follow the same reporting timeframe as initial reports.

Pregnancy Reporting:

Pregnancies reported during study conduct should be promptly reported to the sponsor or designee as soon as the investigator is notified, using the Pregnancy Reporting Form.

Transmission of SAE Reports and Pregnancy Forms:

Completed SAE reporting forms and completed pregnancy forms should be transmitted to the sponsor or designee as described in the X-22-201 safety monitoring plan.

IRB/Ethics Reporting Requirement:

The Investigator must comply with the applicable IRB or Ethics Committee requirements for reporting of SAEs to the IRB.

Follow-up of AEs and SAEs:

All AEs and SAEs must be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or the subject is lost to follow-up. The investigator is responsible for ensuring that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. Once resolved, the appropriate AE/SAE CRF page(s) should be updated. If a subject dies during participation in the study or within 30 days of participation in the study, a copy of any post-mortem findings, including histopathology, should be obtained, if available, and forwarded to the Sponsor or designee.

12.3.4.7. IND Safety Reports

This protocol has been filed under an Investigational New Drug (IND) application with the U.S. FDA Under 21 CFR 312.32 (c). The sponsor is required to notify FDA and all participating investigators in an IND Safety Report of potentially serious risks from clinical trials or any other source, as soon as possible, but no later than 15 calendar days after receiving the safety information and determining that the information qualifies for reporting. As defined below, the sponsor must report in an IND Safety Report any “suspected adverse reaction” that is both “serious” and “unexpected”, as defined above.

12.3.4.8. Unanticipated Problems and Notification

The investigator must notify the sponsor of designee within 24 hours of awareness of all unanticipated problems involving the risk to subjects and others. For the purposes of this study, an “Unanticipated Problem” is defined as any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity or frequency (i.e., not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.).

- Related or possibly related to participation in the research (i.e., possibly related means there is a reasonable possibility that the incident experience or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic or social harm).

12.4. Appropriateness of Measurements

All assessments to be used in this study are commonly used, standard measurements frequently utilized in the evaluation of smoking cessation.

13. DATA MANAGEMENT AND STATISTICAL CONSIDERATIONS

Completed CRFs for this study will be forwarded to the sponsor or its representative where editing and construction of a quality-assured database will occur. The statistical analysis of these data will be performed by the sponsor or its representative. The statistical evaluation will be performed using the Statistical Analysis Software (SAS®) Version 9.1 (SAS Institute, Cary, NC) or higher. All data will be listed and summary tables will be provided.

13.1. Determination of Sample Size

The statistical framework will be to demonstrate a significant improvement in the proportion of subjects on active treatment (X-22 Smoking Cessation Product) that achieves continuous abstinence from smoking within Weeks 7 to 10 as compared to the control group. As such, the null and alternative hypotheses to be tested are as follows:

H0: $P_{active} = P_{control}$

H1: $P_{active} > P_{control}$

Assuming a response rate of 15% in the control arm and at least a 35% response rate in the active arm, a sample size of 216 subjects (108 in each arm) is sufficient to achieve at least 92% power to establish superiority using a 5% two-sided significance level. The sample size calculation is based on the Chi-Square test.

Table 13-1 shows the power for testing rate over a range of possible control group responses and estimated X-22 treatment effects assuming a total sample size of 216 and a 5% two-sided significance level.

Table 13-1 Power to Detect Treatment Effect on Response of Active Group Given Sample Size of 216

Control Response	Incremental Increase in Response in the Active Group Compared with Control			
	10%	15%	20%	25%
10%	53%	83%	96%	99%
12%	49%	79%	94%	99%
15%	45%	75%	92%	98%
18%	41%	71%	91%	98%
20%	39%	69%	89%	97%

13.1. Study Populations

The Safety population includes all subjects who have received at least one dose of investigational product.

The intent to treat (ITT) population includes all subjects who are randomized.

The Per Protocol (PP) population is defined as those randomized subjects who have completed the study without a major protocol violation.

Subjects will be analyzed according to the treatment received. Safety will be evaluated using the safety population. Efficacy will be evaluated using the ITT and PP populations.

13.2. General Consideration for Data Analysis

In general, descriptive summaries will include n, mean, standard deviation, median, minimum, and maximum for continuous variables and n and percent for categorical variables. Summaries will present data by treatment group and where appropriate, by assessment time.

Missing values will not be imputed. For the analyses of categorical efficacy data (e.g., percentage of subjects archive continuous abstinence of smoking), any subject that is missing data due to premature discontinuation of the study will be considered a failure at the time points on or following the date of discontinuation.

13.3. Background and Demographic Characteristics

Demographic and baseline measurements will be summarized using standard descriptive methods by treatment arm and overall. Demographic summaries will include gender, self-identified race/ethnicity (with Asian-Chinese, Asian-Indian, Asian-Other combined to Asian), and age. Baseline characteristic summaries will include body weight, height, body mass index, and additional parameters as necessary.

Homogeneity of these variables will be assessed by t-test for continuous measures and by chi-square tests for categorical measures. If any significant differences ($p<0.10$) are found between treatment arms, additional exploratory analyses will be performed to assess the impact of this lack of baseline homogeneity on relative treatment effects.

13.4. Efficacy Analysis

The primary endpoint, 4 weeks of continuous abstinence (Weeks 7-10), will be assessed after treatment termination, at Visits 6 and 7. Abstinence rates will be-verified by exhaled CO <10 ppm for each subject. This endpoint will be analyzed using a Cochran-Mantel-Haenszel (CMH) test statistic stratified by gender. The 95 percent confidence intervals of the 4 weeks of continuous abstinence will also be provided. A Logistical regression analyses will also be performed to evaluate the impact of demographics and baseline characteristics on the 4-week continuous abstinence rate.

Secondary efficacy endpoints include:

- Abstinence rates at 2 and 3 months following treatment termination: 7-day smoking abstinence rates at 2 and 3 months following treatment termination will be assessed by self-reports and verified by exhaled CO <10 ppm at Visits 8 and 9.
- Withdrawal symptoms: Withdrawal symptoms will be assessed by subjective questionnaire using The Minnesota Nicotine Withdrawal Scale.
- Cigarette consumption during the treatment period: Cigarette consumption will be assessed by the number of cigarettes smoked according to smoking diaries, exhaled CO levels, and salivary cotinine levels.

The abstinence rates will be analyzed similarly as the primary endpoint at each time point. The abstinence rates over time will be compared between treatment groups using a generalized estimating equation (GEE) model. The comparisons between treatment groups on cigarette consumption will be performed using repeated measure of analysis of covariance (ANCOVA). Demographics and baseline characteristics will be included as covariates of interest. Severity of withdrawal symptoms will be compared between treatment using Chi-square text or Fisher's exact test.

Exploratory endpoints will include:

- Results of the Sensory Questionnaire.
- Results of the Fagerström Test of Nicotine Dependence.
- Accuracy of subjects' guesses at Visit 7 regarding which treatment group they were assigned to.
- Cotinine levels at each study visit.

The exploratory endpoints will be summarized by treatment group. Statistical models may be used to explore the between treatment differences.

The details of the statistical analyses will be included in the statistical analysis plan.

13.5. Safety Analysis

Safety and tolerability endpoints will be analyzed by descriptive statistics. No formal statistical comparisons will be performed.

Adverse event verbatim text will be coded and classified by body system and preferred (coded) term using the Medical Dictionary for Regulatory Activities (MedDRA) or current coding system. The relationship of AE body systems, preferred terms, and verbatim text will be listed. All AEs will be listed. Summaries of AEs will be presented by treatment group for All AEs, Drug-related AEs, SAEs, and AEs leading to study/investigational product discontinuation.

Individual data for vital signs measurements, weight, and physical examination findings will be listed by subject and summarized for each treatment arm by incidence of events/abnormalities or descriptive statistical summaries (n, mean, SD, median, Q1, Q3, minimum, and maximum), as appropriate.

Clinical laboratory values will be listed and summarized by treatment and time of assessment. Laboratory values that fall outside the reference range will be flagged.

Concomitant medications will be split into 3 categories: previous medications (medications administered before and only before study treatment start), concomitant medications (medications administered during study treatment) and post-medications (medications started after study treatment stop).

13.6. Rules for Excluding Subjects from Analysis

Protocol violations are defined as deviations from the procedures outlined in the protocol. All decisions regarding the type of deviations (major or minor) will be discussed and agreed in a meeting prior to unblinding of the study. Individuals having any major deviations will not be included in the PP population.

A listing of all subjects with protocol deviations will be presented. A data listing presenting the eligibility for the analysis populations for each subject will also be presented. The eligibility will be based on the decisions agreed in the review meeting regarding the major protocol deviations that constitute protocol violations.

14. STUDY MANAGEMENT

14.1. Approval and Consent

14.1.1. Regulatory Guidelines

This study will be performed in accordance with United States investigational new drug (IND) regulations (21 Code of Federal Regulations [CFR] 56), the guidelines of the International Conference on Harmonisation (ICH), and the most recent guidelines of the Declaration of Helsinki (Appendix 2).

14.1.2. Institutional Review Board/Independent Ethic Committees

Conduct of the study must be approved by an appropriately constituted institutional review board (IRB). Approval is required for the study protocol, investigational drug brochure, protocol amendments, informed consent forms, subject information sheets, and advertising materials. The study will not commence until written IRB authorization has been received by the sponsor or its representative.

14.1.3. Informed Consent

For each trial subject, written informed consent will be obtained prior to any protocol-related activities. As part of this procedure, the principal investigator or one of his/her associates must explain orally and in writing the nature, duration, and purpose of the study, and the action of the investigational product in such a manner that the subject and (if applicable) appointed guardian are aware of the potential risks, inconveniences, or adverse effects that may occur. They should be informed that the subject may withdraw from the study at any time. They will receive all information that is required by federal regulations and ICH guidelines. The principal investigator will provide the sponsor or its representative with a copy of the IRB-approved informed consent form prior to the start of the study.

14.2. Discontinuation of the Study by the Sponsor

The sponsor reserves the right to discontinue the study at this site or at multiple sites for safety or administrative reasons at any time. In particular, a site that does not recruit at a reasonable rate may be discontinued. Should the study be terminated and/or the site closed for whatever reason, all documentation and study medication pertaining to the study must be returned to the sponsor or its representative.

14.3. Study Documentation

By signing a copy of Form FDA 1572, the principal investigator acknowledges that he/she has received a copy of the Investigator's Brochure on X-22 Smoking Cessation Product and assures the sponsor that he/she will comply with the protocol and the provisions stated in Form FDA 1572. No changes in this protocol can be made without the sponsor's written approval.

The investigator will supply the sponsor with the following documents:

- Original, signed Form FDA 1572
- Curricula vitae for all investigators listed on Form FDA 1572
- Copy of principal investigator's state medical license
- Signed protocol signature page
- List of IRB members and their occupations/affiliations or multiple assurance number
- Letter indicating IRB approval to conduct the protocol
- Copy of IRB-approved informed consent form
- Disclaimer if the investigator is an IRB member

The sponsor will supply the investigator with the following documents:

- Clinical study protocol
- Investigational product brochure
- Sample informed consent form
- CRFs/instruction manual
- Laboratory certification records and reference ranges

14.4. Data Handling

CRFs must be completed for every subject who signs an ICF and has screening procedures performed. The investigator must ensure the accuracy, completeness, legibility, and timeliness of data reported in the CRF and all required reports. Any change or correction to a CRF must be dated, initialed, and explained (if necessary), and must not obscure the original entry. This process applies to both written and electronic changes.

Data reported on the CRF that are derived from source documents should be consistent with the source documents, or the discrepancies must be explained.

Within (1 week or other agreed timeframe) of completion of each subject, the investigator should agree to have completed and signed CRFs available for inspection by the clinical monitor.

14.5. Study Monitoring and Auditing

This study will be monitored at all stages of its development by the clinical research personnel employed by the sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to protocol and in order to comply with guidelines of good clinical practice. On-site review of CRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each subject. Note that a variety of original documents, data, and records will be considered as source documents in this trial. The CRF itself is not to be used as a source document under any circumstances.

Medical advisors and clinical research associates or assistants may request to witness subject evaluations occurring as part of this protocol. The investigator and appropriate personnel will be periodically requested to attend meetings/workshops organized by the sponsor to assure acceptable protocol execution. The study may be subject to audit by the sponsor or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required subject records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in CRF generation, where clinically appropriate.

14.6. Retention of Records

The investigator agrees to comply with all applicable federal, state, and local laws and regulations relating to the privacy of patient health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR, Parts 160 and 164 (the Health Insurance Portability Accountability Act of 1996 [HIPAA] Privacy Regulation). The investigator shall ensure that study subjects authorize the use and disclosure of protected health information in accordance with HIPAA Privacy Regulation and in a form satisfactory to the sponsor.

The investigator must arrange for retention of study records at the site for 2 years after the study medication's new drug application (NDA) is approved or IND is withdrawn, as required by FDA regulations. The investigator should take measures to prevent accidental or premature destruction of these documents.

14.7. Use of Study Findings

By signing the study protocol, the investigator agrees to the use of results of the study for the purposes of national and international registration. If necessary, the authorities will be notified of the investigator's name, address, qualifications, and extent of involvement. Reports covering clinical and biometric aspects of the study will be prepared by the sponsor or its representative.

14.8. Publication

As a multicenter trial, the sponsor intends to publish clinical data from all centers participating in the investigation. A publication committee selected by the sponsor will submit draft manuscripts to all participating investigators for their comments. In conformity with the uniform requirements for manuscripts submitted to biomedical journals published by the International Committee of Medical Journal Editors,[33] investigators whose contribution consists solely in the collection of data will not be named individually as authors. Rather, those investigators will receive a collective authorship as the "X-22 Study Group" and will be identified in a note.

Individual investigators and/or their associates subsequently may publish additional findings of this study in scientific journals or present them at scientific meetings, provided that the sponsor is given ample opportunity to review any proposed abstract, manuscript, or slide presentation prior to its submission. This review is required to ensure that the sponsor is aware of all written and oral presentations of the data and does not imply any editorial review or restriction of the contents of the presentation or use.

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16. Appendices

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Appendix 1 Schedule of Study Visits and Assessments

	Screen	Treatment							Abstinence				Follow-up		
	Visit 1	Visit 2	TC [e]	Visit 3	TC [e]	Visit 4	TC [e]	Visit 5 Quit [h]	TC [e]	Visit 6	TC [e]	Visit 7/ ET [j]	TC [e]	Visit 8 [n]	Visit 9
Assessments	Day -7	Day 0 ±2 days	Wk 1 ±2 days	Wk 2 ±2 days	Wk 3 ±2 days	Wk 4 ±2 days	Wk 5 ±2 days	Wk 6/ ±2 days	Wk 7 ±2 days	Wk 8 [i] ±2 days	Wk 9 ±2 days	Wk 10 [k]/ +4 days	Wk 13 [m] +3 days	Wk 14/ ±2 days	Wk 18/ ±2 days
Informed Consent	X														
Inclusion/Exclusion Criteria	X														
Plan to Quit Smoking Query	X														
Urine Drug Screen	X														
Clinical Laboratory Tests [a]	X							X				X(ET only)[l]			
Electrocardiogram	X														
Demographics	X														
Medical History	X														
Smoking History	X														
Dispense Behavioral Support [b]						X		X		X					X
Concomitant Medications	X	X		X		X		X		X		X		X	X
Physical Examination [c]	X											X			
Urine Pregnancy Test [d]	X											X			
Alcohol Intake	X														
Blood Pressure and Heart Rate	X	X		X		X		X		X		X		X	X
Height	X														
Weight	X	X		X		X		X		X		X		X	X
Carbon Monoxide Level	X	X		X		X		X		X		X		X	X
Saliva Cotinine Level	X	X		X		X		X		X		X		X	X
Randomization		X													
Sensory Questionnaire	X	X		X		X		X							
Withdrawal Questionnaire	X	X		X		X		X		X		X		X	X
FTND	X	X		X		X		X							
Self-report Diary	X	X		X		X		X		X		X		X	X
Dispense Study Product		X		X		X									
Return Unused Study Product				X		X		X							
Check Supply of Study Product			X		X		X								
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Telephone Call [e]			X		X		X		X		X		X		
Treatment Guess [f]												X			
Smoking Abstinence Query [g]										X		X	X	X	X

AE=adverse events; ET=early termination; FTND= Fagerström Test of Nicotine Dependence ; TC=telephone call; Wk=week

[a] Complete blood count and differential and clinical chemistries.

[b] Behavioral support includes ~15-minute smoking cessation counseling sessions and supporting printed materials appropriate to the phase of the study.

[c] Physical examination includes measurement of respiratory rate and temperature.

[d] Woman of childbearing potential only.

[e] The site will call each subject between site visits During the Treatment Phase at Weeks 1, 3, and 5 to ensure that the subject has an adequate supply of investigational product and to obtain information regarding AEs. During the Abstinence Phase, at Weeks 7 and 9, the site will call each subject to obtain information regarding AEs. During Follow-up, at Week 14, the site will call each subject and question them about their smoking status; subjects who have smoked will be questioned about AEs; subjects who have not smoked within the past 7 days will be asked to come to the site within 1 week. The site will also call each subject the day before each site visit to remind the subject of their appointment.

[f] Each subject will be asked to guess which treatment group they were assigned to.

[g] All subjects will be asked if they have smoked since the last visit and if they have smoked within the last 7 days.

[h] Visit 5 is the "Quit Date" (all subjects quit smoking).

[i] Visit 6/Week 8 must occur between 12 to 16 days after the Quit date.

[j] Visit 7 assessments will also be performed for subjects who terminate the study early.

[k] Visit 7/Week 10 must occur between 28 to 32 days after the Quit date.

[l] Subjects who drop out of the study prior to Visit 5 will also have laboratory studies completed at the Early Termination Visit. Subjects who have completed Visit 5 do not need additional laboratory studies completed at Visit 7.

[m] The Week 13 telephone call must take place 4 to 7 days prior to Week 14.

[n] Only subjects who report during their Week 13 telephone call that they have not smoked in the previous week will complete Visit 8.

Appendix 2 World Medical Association Declaration of Helsinki

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best

current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the

research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders

them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

- The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
- Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

Informed Consent Form

Important

You are being asked to participate in a research study. The purpose of this form is to provide you with information to consider in deciding whether to be in this research study. Your consent should be made based on your understanding of the nature and risks of the treatment.

Please ask questions if there is anything you do not understand. Please ask the study doctor or the study staff to explain any words or information not clear to you.

Also, you should have your personal treating physician call the study doctor to ask any questions he or she feels are necessary to evaluate the study and your possible participation in it.

You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

Your participation is voluntary and will have no effect on the quality of your medical care if you choose not to participate.

1. Who will be conducting the study?

The following doctors will be the principal investigators for this study:

[To be completed for each study site]

2. Who is sponsoring the research project?

This study is sponsored by a company named 22nd Century Limited, LLC.

3. Where will the study be conducted?

This study will be conducted at

[To be completed for each study site]

4. What is the purpose of the research?

22nd Century has begun a research study of a product called X-22 Smoking Cessation Product. X-22 Smoking Cessation Product is being studied to help people who are addicted to cigarettes quit smoking. X-22 cigarettes look like regular cigarettes, and like regular cigarettes, they consist of filler, a filter, and a wrapper. X-22 cigarettes contain menthol, (b) (4) The filler in X-22 cigarettes, like regular cigarettes, is made of cured tobacco leaf, except that the tobacco used for X-22 cigarettes is from a tobacco plant that has very low levels of nicotine. X-22 cigarettes have a tobacco content per cigarette and tar yield similar to regular cigarettes, but have a low nicotine content and a nicotine yield that is lower by more than 95% compared to regular cigarettes. Because of that it is called a very low nicotine, or "VLN" cigarette. The smoke from X-22 cigarettes is very similar to the smoke from regular cigarettes.

X-22 is an investigational product. An investigational product is one that has not been approved by the Food and Drug Administration (FDA). You are being asked to participate in this study because you are a smoker who has plans to quit smoking.

This study involves coming to the study site at least 8 or 9 times. On the first visit, the doctor will do tests to see if you can be in the study. If you and the doctor both decide that you should be in the study, then you will come back a week later and the doctor will give you study cigarettes to begin the Treatment Phase. You will smoke these cigarettes and no other cigarettes for 6 weeks and come to the study site every 2 weeks for tests. After 6 weeks, the Abstinence Phase begins and you will quit smoking and continue to come to the study site for tests every 2 weeks for the next month. The last 2 months of the study are the Follow-up Phase. During this time you will come to the study site every 4 to 8 weeks. It is important that you do not use any tobacco products other than the study cigarettes or use any products containing nicotine (for example nicotine gum such as Nicorette®, patches such as Nicotrol® or Nicoderm®, inhalers such as Nicotrol®, or sprays such as Nicotorol NS®) or drug therapy that may aid in smoking cessation (for example Chantix®, Zyban®, or

Welbutrin®) during the study. It is also important that you do not use any alternative smoking cessation therapies during this study such as hypnosis, herbal therapy, alternative therapies, or acupuncture. You will also have 4 smoking cessation counseling sessions of about 15 minutes each during the study.

X-22 has not been studied in people before now, but another kind of cigarette (brand name Quest®) that was made with the same kind of tobacco as X-22 was sold in the United States from 2003 to 2007 and several research studies involving about 1000 people were done using Quest cigarettes. These studies showed that if smokers smoke VLN cigarettes for at least 6 weeks before quitting smoking, they have less cravings and fewer other withdrawal symptoms when they quit. This is because smoking the VLN cigarette gives similar tastes and behaviors to regular cigarettes, but does not deliver a satisfying amount of nicotine. This breaks the link between nicotine and other satisfying parts of smoking and makes it easier to quit.

5. Who is being asked to participate in this research study?

All drug research studies have guidelines about who can be in the study. These guidelines have to do with things such as age, medical history, smoking history, and how healthy you are. To qualify for this research study you must be a healthy smoker who is planning to quit smoking. The study doctor will use standard tests to see if you meet the requirements.

You may not be allowed to participate if you have certain other diseases or illnesses, if you need to take certain medications, or if you have certain diseases in your history. Even if you meet other qualifications for taking part in the study, you will not be allowed to take part if the study doctor determines that you have any condition that makes it unsafe or not appropriate for you to be in the study.

6. What do I have to do?

During the study you will have the following responsibilities:

- Attend all scheduled visits.
- During the Treatment Phase, smoke the study cigarettes as you would normally smoke, and do not use any other tobacco products or other products containing nicotine.

- Quit smoking at the end of the Treatment Phase.
- Notify the study doctor of any illnesses or injuries, unexpected or troublesome side effects, or problems that occur during the study.
- Notify the study doctor if you plan to have an elective surgery or any other medical treatment or procedure.
- Arrange for routine medical and psychiatric care with a private medical doctor, since being in the study does not take the place of regular medical care.
- Make sure that the study cigarettes are kept out of the reach of children and do not let anyone else smoke them. You are the only person who should use the study cigarettes.

7. What procedures will be performed for research purposes?

At the first visit, the study doctor will perform examinations and tests to decide if you should take part in the study. The following tests and examinations will be performed:

1. An interview to obtain a medical and smoking history.
2. A physical examination.
3. An electrocardiogram (ECG—a recording of the electrical activity of your heart).
4. Laboratory tests—about 4 teaspoons of blood will be taken from your arm using a small needle, and you will be asked to give a urine sample. Your blood will be tested for levels of certain components to see if they are normal. Your urine will be tested for drugs, and if you are a woman, your urine will be tested to see if you are pregnant.
5. A recording of your blood pressure, pulse, respiratory rate, height, and weight.
6. Carbon dioxide (CO) concentrations will be measured: You will breathe into a machine to measure how much CO you exhale.
7. Cotinine measurements: About 80 percent of the nicotine you take in from smoking or using other nicotine products is made into cotinine by your body. To measure how much cotinine is in your body, you will spit into a tube and the saliva will be tested.
8. You will be asked to fill out 3 questionnaires to find out how much you liked the last cigarette you smoked and how dependent your body is on smoking.

9. You will be given a diary card and asked to write down how many cigarettes you smoke for the next week and if you use any other tobacco or nicotine products.

If you are approved for entry into the study you will make an appointment to come back to the study site in 1 week. At the second study visit, more tests will be done and a 2-week supply of study cigarettes will be given to you along with instructions on filling out your diary card. You will be given enough cigarettes to last until the next scheduled visit, but if you run out, you must call the study site to get more study cigarettes. It is important that you do not smoke any other cigarettes during the study.

If you are accepted into the study, you will be assigned by chance, like the flip of a coin, to one of the following treatments:

- ***X-22 Cigarettes***
- ***Regular Cigarettes that look just like X-22 cigarettes and also contain menthol***

The chance of your being assigned to either of these treatments is 1 in 2. You will not be told which treatment you are receiving. Neither the study doctor nor any other person involved in the study will know whether you are receiving X-22 or regular cigarettes. However, this information will be made available to the study doctor if it becomes medically necessary.

You will visit the clinic for a total of 8 to 9 visits over the 19 week study period. At these visits, the study doctor or the study doctor's staff will again perform certain tests and procedures and interview you about how you are feeling.

Below is a list of these procedures with the minimum number of visits each kind of procedure will be performed:

Procedure	Number of Visits
1. Physical examination	2
2. Measurement of blood pressure, pulse rate, and weight	8 or 9
3. Electrocardiogram	1
4. Laboratory tests involving your blood, requiring the drawing of about 4 teaspoons of blood from your arm using a small needle	2
5. Laboratory tests involving your urine, requiring you to provide a small urine sample	1 (men) 2 (women)
6. Interview about how you are feeling. Some visits will include a series of tests that involve specific questions about your progress.	8 or 9
7. Measure CO levels that you exhale	8 or 9
8. Measure cotinine in your saliva	8 or 9
9. Smoking cessation counseling sessions	4
10. Fill out questionnaires (1 to 3 questionnaires depending on which study visit)	8 or 9

The visits will last from 15 minutes to 2.5 hours.

8. What are the possible risks, side effects, and discomforts of this research study?

- Like regular cigarettes, X-22 cigarettes are not safe and pose health risks to subjects, namely cancer, cardiovascular disease, respiratory disease, and harm to an unborn baby in mothers who smoke. Unknown risks and side effects are also possible.

- Smoking cigarettes can cause blood clots to form in ways that cause diseases of the heart and blood vessels. One research group has studied the effect of cigarette smoke on platelets, a kind of cell in the blood that is involved in making blood clots. This group has shown that cigarette smoke with no nicotine or low nicotine in it may cause more changes to platelets than cigarette smoke with regular amounts of nicotine. It is not known, but it is possible, that these changes to platelets could cause an increased risk of blood clots forming in people who smoke VLN cigarettes.
- Taking blood from your arm may cause swelling, pain, redness, bruising, or infection (infection rarely happens) at the site where the needle is inserted.
- You may experience mild irritation, slight redness, or itching at the sites on your skin where electrodes are placed for the ECG measurements.
- After the Treatment Phase you will be asked to quit smoking. Many people experience cravings and other unpleasant withdrawal symptoms when they quit smoking.
- If you are nursing an infant or you are pregnant now, you must not be in the study because smoking cigarettes, including X-22, is harmful to a fetus. If you are sexually active and are at risk of getting pregnant, you and your male partner(s) must use a method of birth control that works well, like birth control pills, Depo-Provera, Norplant, an IUD, a diaphragm or condom with spermicide, or abstinence. You will have to do this the whole time you are in this study. If you become pregnant during the research study, please tell the study doctor or staff immediately.

9. What are the possible benefits to taking part in this research study?

There is no guarantee that you will receive any benefits from this study. However, if you are able to quit smoking during the study, that would be beneficial to your health. You may also feel that you are benefiting in the following ways:

- Your health will be monitored as long as the study lasts. Services provided and evaluations performed as a part of the study are limited, however, and should not be considered a substitute for a thorough evaluation, ongoing medical care, or follow-up by your personal doctor.
- You will be helping others by contributing to medical research.

10. What treatments or procedures are available if I decide not to participate in this research study?

You do not have to take part in this study to quit smoking. Other smoking cessation programs are available in your community. Your personal physician or the study doctor can answer any questions you have about other smoking cessation treatments and programs.

11. Will I be told of any new information or new risks that may be found during the course of this study?

You will be notified of any significant new findings that may cause you to change your mind about participating in the research study.

12. Will my insurance provider or I be charged for any costs of any procedures performed as part of this research study?

There are no costs to you associated with this study.

13. Who will pay if I am injured as a result of taking part in this research study?

If you require medical treatment for an illness or injury that is a direct result of taking part in this study, 22nd Century will pay for reasonable and customary costs of such treatments if the following conditions are met:

- The illness or injury was a result of study participation.
- The cost of treatment or any part of the costs is not covered by any other health insurance, government health program, or others providing coverage for health care.

Neither financial compensation nor reimbursement for such things as pre-existing conditions, illnesses or diseases totally unrelated to the study, lost wages, property damage, disability, or discomfort is available. It is your obligation, if you feel impaired in any way, to stop activity that may cause injury to yourself, to any other person, or to property as a result of participating in this study (e.g., driving a vehicle, operating machinery, climbing a ladder).

You or your insurance company will be responsible for payment for any tests or care done outside of the scope of the study.

14. Will I be paid for participating in this study?

22nd Century appreciates your participation in this study. You will be compensated for your time and the inconvenience of being in the study. You will only be paid for the visit(s) that you complete. A schedule of payments is given below:

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Total
$50.00	$75.00	$75.00	$75.00	$75.00	$75.00	$75.00	$75.00	$75.00	**$650.00**

15. Who will know about my participation in this research study?

Information related to you will be treated in strict confidence to the extent provided by law. Your identity will be coded and will not be associated with any published results. Your code number and identity will be kept in a locked file of the study doctor.

In order to monitor this research study, medical records that identify you and the consent form signed by you may be inspected and/or copied by the following parties:

- 22nd Century Limited, LLC (the Sponsor)
- Any other agent of the Sponsor

Information from this study will be submitted to 22nd Century and to the FDA. This information may also be submitted to governmental agencies in other countries where X-22 may be considered for approval.

In addition, your medical records may be inspected by the following parties:

- FDA
- Department of Health and Human Services (DHHS) agencies
- Governmental agencies in other countries
- Copernicus Group Institutional Review Board (IRB), a group that reviews research studies

Because of the need to release information to these parties, absolute confidentiality cannot be guaranteed. The results of this research study may be presented at meetings or in publications; however, your identity will never be disclosed in those presentations.

Any disclosure of results required by the FDA may reveal your identity. There are, however, limited circumstances that would allow the FDA to require such disclosure. Any disclosure of results to pharmaceutical companies may reveal your identity. However, your records containing your name will not be removed from the study doctor's office nor will any copies of these records with your name be made. Any records or data sent to pharmaceutical companies will have your identity protected by subject code and will not contain your name.

A description of this clinical trial will be available on http://www.clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

While this study is still in progress, you may not be given access to medical information about you that is related to the study. After the study is completed and the results have been analyzed, you will be permitted access to any medical information collected about you in the study.

You have the right to revoke this authorization and can withdraw your permission for us to use your information for this research by sending a written request to the study doctor listed on page two of this research consent form. If you do send a letter to the study doctor, the use and disclosure of your protected health information will stop as of the date that he or she receives your request. However, the study doctor is allowed to use and disclose information collected before the date of the letter or collected in good faith before your letter arrives. Revoking this authorization will not affect your health care.

By signing this consent form, you (or your legally acceptable representative) authorize access to this confidential information.

16. Is my participation in this research study voluntary?

Yes. Your participation in this study is voluntary and you may stop your participation at any time. You will not be penalized in any way nor will there be any penalty or loss of

benefits to which you are entitled at this site if you decide not to participate or you leave the study.

If you decide to leave the study, you should contact the study doctor who will explain the best way for you to quit. The study doctor may ask you to complete additional tests and examinations and will explain the reason for these tests. You should also contact your personal physician so he or she can provide you with the best course of continuing care, including assistance with other smoking cessation treatments.

17. Can I be removed from the study without my consent?

Yes. The study doctor or 22nd Century can remove you from the study without your consent, for any reason. Possible reasons for doing so include the following:

- Any change in your medical condition that might be harmful to you.
- Your failure to follow the study doctor's instructions.
- Administrative purposes.
- Termination of the study.

18. What if I have a question or problem?

You should feel free to ask questions about the study and your rights as a subject before, during, and after the study.

Who to Call

If you have any questions about this research study or if at any time you believe that you have a research-related injury or a reaction to the study medication, you should contact (*insert name and phone number of investigator for each study site*)

If you have any questions regarding your rights as a research subject, you may contact:

Copernicus Group IRB
1 Triangle Drive, Suite 100
Durham, NC 27713
Phone: 888-303-2224

Important

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Signing your name to this consent form means that you voluntarily agree to take part in this study.

This agreement can be withdrawn at any time.

Consent To Participate

I have read and I understand this consent form. I have also been given the opportunity to ask any questions I had about the study, and all questions I asked were answered to my satisfaction. I understand the risks and benefits of taking part in this study as described in this consent form. I freely consent to be treated with X-22 Smoking Cessation Product under the study doctor's direction. To the best of my knowledge, I also certify that all information I have given about my medical history is true and correct.

I understand that I am free to withdraw from the study at any time. I will notify the study doctor if I decide to withdraw so that my participation may be ended in an orderly manner and my future care can be discussed.

I understand that I will be informed of any information that might relate to my willingness to continue in the study.

If I have any physical or psychiatric symptoms or problems, I will tell the study doctor.

I understand that I will receive a signed and dated copy of this consent form for my records.

[Include for State of California study site only:] I acknowledge that I have received a copy of the **California Experimental Subject's Bill of Rights**.

I authorize the release of my medical records, including the results of urine drug screens to 22nd Century and their agents, the FDA, DHHS agencies, governmental agencies in other countries, and the Copernicus Group IRB.

My consent to participate in this research study does not take away any legal rights in the case of negligence or other legal fault of anyone who is involved with this study. Nothing in this consent form is intended to change any applicable federal, state, or local laws regarding informed consent.

_____
Name of Participant (Print)

_____ _____
Signature of Participant Date

_____ _____
Signature of Study Doctor or Person Administering Consent Date

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

STATEMENT OF INVESTIGATOR

(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)

(See instructions on reverse side.)

Form Approved: OMB No. 0910-0014.
Expiration Date: May 31, 2009.
See OMB Statement on Reverse.

NOTE: No investigator may participate in an investigation until he/she provides the sponsor with a completed, signed Statement of Investigator, Form FDA 1572 (21 CFR 312.53(c)).

1. NAME AND ADDRESS OF INVESTIGATOR

Laurence Chu, MD
Benchmark Research
1015 East 32nd St, Suite 309
Austin, TX 78705

2. EDUCATION, TRAINING, AND EXPERIENCE THAT QUALIFY THE INVESTIGATOR AS AN EXPERT IN THE CLINICAL INVESTIGATION OF THE DRUG FOR THE USE UNDER INVESTIGATION. ONE OF THE FOLLOWING IS ATTACHED.

☒ CURRICULUM VITAE ☐ OTHER STATEMENT OF QUALIFICATIONS

3. NAME AND ADDRESS OF ANY MEDICAL SCHOOL, HOSPITAL, OR OTHER RESEARCH FACILITY WHERE THE CLINICAL INVESTIGATION(S) WILL BE CONDUCTED.

Benchmark Research
1015 East 32nd St, Suite 309
Austin, TX 78705

4. NAME AND ADDRESS OF ANY CLINICAL LABORATORY FACILITIES TO BE USED IN THE STUDY.

Spectra Clinical Research
8 King Road
Rockleigh, NJ 07647

5. NAME AND ADDRESS OF THE INSTITUTIONAL REVIEW BOARD (IRB) THAT IS RESPONSIBLE FOR REVIEW AND APPROVAL OF THE STUDY(IES).

Copernicus Group IRB
1 Triangle Drive, Suite 100
Durham, NC 27713

6. NAMES OF THE SUBINVESTIGATORS *(e.g., research fellows, residents, associates)* WHO WILL BE ASSISTING THE INVESTIGATOR IN THE CONDUCT OF THE INVESTIGATION(S).

Roberta Melina Braun, MD, FACOG
Lamar Box, CCRC
Michelle Listz, CRC
Ann Rutledge, MA, CCRC
Francesca Vigil, CCRC
Kay Smith, CCRC
Lauren Christal, CCRC
Pamela Conley, MA, CRC

7. NAME AND CODE NUMBER, IF ANY, OF THE PROTOCOL(S) IN THE IND FOR THE STUDY(IES) TO BE CONDUCTED BY THE INVESTIGATOR.

A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid X-22-201 (IND# 103,589)

PSC Graphics (301) 443-1090 EF

8. ATTACH THE FOLLOWING CLINICAL PROTOCOL INFORMATION:

☐ FOR PHASE 1 INVESTIGATIONS, A GENERAL OUTLINE OF THE PLANNED INVESTIGATION INCLUDING THE ESTIMATED DURATION OF THE STUDY AND THE MAXIMUM NUMBER OF SUBJECTS THAT WILL BE INVOLVED.

☒ FOR PHASE 2 OR 3 INVESTIGATIONS, AN OUTLINE OF THE STUDY PROTOCOL INCLUDING AN APPROXIMATION OF THE NUMBER OF SUBJECTS TO BE TREATED WITH THE DRUG AND THE NUMBER TO BE EMPLOYED AS CONTROLS, IF ANY; THE CLINICAL USES TO BE INVESTIGATED; CHARACTERISTICS OF SUBJECTS BY AGE, SEX, AND CONDITION; THE KIND OF CLINICAL OBSERVATIONS AND LABORATORY TESTS TO BE CONDUCTED; THE ESTIMATED DURATION OF THE STUDY; AND COPIES OR A DESCRIPTION OF CASE REPORT FORMS TO BE USED.

9. COMMITMENTS:

I agree to conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.

I agree to personally conduct or supervise the described investigation(s).

I agree to inform any patients, or any persons used as controls, that the drugs are being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

I agree to report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with 21 CFR 312.64.

I have read and understand the information in the investigator's brochure, including the potential risks and side effects of the drug.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

I agree to maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.

I will ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312.

INSTRUCTIONS FOR COMPLETING FORM FDA 1572
STATEMENT OF INVESTIGATOR:

1. Complete all sections. Attach a separate page if additional space is needed.
2. Attach curriculum vitae or other statement of qualifications as described in Section 2.
3. Attach protocol outline as described in Section 8.
4. Sign and date below.
5. FORWARD THE COMPLETED FORM AND ATTACHMENTS TO THE SPONSOR. The sponsor will incorporate this information along with other technical data into an Investigational New Drug Application (IND). INVESTIGATORS SHOULD NOT SEND THIS FORM DIRECTLY TO THE FOOD AND DRUG ADMINISTRATION.

10. SIGNATURE OF I (b) (6) [redacted]	11. DATE
	11 APRIL 2011

(**WARNING:** A wil [redacted] tle 18, Sec. 1001.)

Public reporting burden for this collection of information is estimated to average 100 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Office of the Chief Information Officer (HFA-710)
5600 Fishers Lane
Rockville, MD 20857

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Please DO NOT RETURN this application to this address.

benchmark research

Laurence Chu, MD, FACS

(b) (6)

Benchmark Research
1015 East 32nd St., Suite 309
Austin, Texas 78705

Private Practice
1015 East 32nd St., Suite 316
Austin, Texas 78705

Licensure:	Current	State of Texas, (b) (6)
Certification:	1994	American Board of Otolaryngology, Board Certified

Medical Education

9/1994-11/1994	USAF School of Aerospace Medicine Flight Surgeon San Antonio, Texas
1992-1993	Emory University Chief Resident Atlanta, Georgia
1988-1993	Emory University Otolaryngology-Head & Neck Surgery Residency Program Atlanta, Georgia
1984-1988	M.D. Degree Boston University School of Medicine Boston, Massachusetts

Undergraduate Education

1982-1988	Bachelor of Arts Boston University Boston, Massachusetts

Professional Experience

2006-Present	Benchmark Research Austin, Texas Investigator
2004-Present	Private Practice Austin, Texas Physician
2000-2004	Ear, Nose & Throat Specialists of Middletown Inc. Middletown, Ohio
1996-2000	Private Practice Southern Illinois ENT Carbondale, Illinois Physician
1993-1996	United States Air Force, Honorable Discharge Otolaryngologist-Head & Neck Surgeon

Professional Memberships

American College of Surgeons, Fellow (1995-Present)

American Academy of Otolaryngology-Head & Neck Surgery, Fellow (1994-Present)

Texas Medical Association (2004-Present)

Travis County Medical Society (2004-Present)

Professional Affiliations

Assistant Clinical Professor of Family Practice
Southern Illinois University-Carbondale (1996-2000)

United States Air Force Physician's Assistant
Specialty Training Program Clinical Instructor (1993-1996)

Publications

Neonatal Laryngoceles: A cause for airway obstruction
Arch Otolaryngol Head Neck Surg. 1994; 120:454-458.

Mandible Fractures: A treatment protocol
J Trauma. 1994;36(1) 45-52.

Selected Clinical Trial Experience

"A Pilot, Multi-Center, Double-Blind, Randomized, Vehicle-Controlled, Parallel-Group Study Comparing XXX and XXX to a Vehicle Control in the Treatment of Acute Otitis Externa"

"A Randomized, Double-Blind, Vehicle-Controlled, Multi-Center, Parallel Group Study to Assess the Efficacy, Safety and Tolerability of XXX Formulation for 24 or 48 Weeks of Treatment in Patients with Mild to Moderate Toenail Onychomycosis"

"A Phase II, Double-Blind, Randomized, Placebo-Controlled, Multi, Dose-Ranging Study of XXX to Assess the Efficacy and Safety of the Vaccine as an Aid to Smoking Cessation"

"A 24-Week, Blinded, Randomized, Placebo-Controlled Dose-Ranging Trial of XXX for Weight Loss in Healthy Obese Patients"

"A randomized, double-blind, placebo-controlled, parallel-group, multicenter study to assess the efficacy ad safety of long-term administration of xxx in the prevention of Type 2 Diabetes in patients with Pre-diabetic status"

"A 2-Year, Randomized, Double-Blind, Placebo-Controlled Phase III Study to Evaluate the Long-Term Efficacy and Safety of XXX in the Treatment of Obese Subjects"

"A Phase III, Randomized, Observer-Blind, Placebo-Controlled, Multi-Center Study to Assess Clinical Efficacy of a XXX Influenza Vaccine and a XXX Influenza Vaccine iin the 2007-2008 Influenza Season in Healthy Adult Subjects"

"A Multi-center, Double-blind, Randomized, Parallel Group Study Evaluating the Therapeutic Equivalence and Safety of XXXX and XXXX and Both Active Treatments to a Vehicle Control in the Treatment of Vulvovaginal Candidiasis Caused by Candida Species"

"A Phase III, Randomized, Placebo-Controlled, Multi-center Trial to Evaluate the Safety and Immunogenicity of a Two-dose Series of XXXX Vaccine Antigen in Association with XXXX in Adults Age 18 or older"

"Phase 3 Double Blind Clinical Study of Effectiveness and Safety of Vero Cell-Derived, Trivalent, Seasonal Influenza Vaccine (VCIV) in Adults 50 Years of Age And Older"

"Double-Blind, Placebo Controlled Phase III Study of the Efficacy of an Investigational Vero Cell-Derived Influenza Vaccine (VCIV) to Prevent Culture Confirmed Influenza Infection (CCII)"

"A Phase III, Observer-Blind, Multi-Centre, Multi-Country, Randomized Study to Evaluate the Immunogenicity and Safety of Thimerosol-Free (TF) XXX Compared with XXX Administered Intramuscularly in Children (6 to 35 months of Age)"

"A Phase III, Observer-Blind, Randomized, Placebo-Controlled, Multi-Center Trial to Evaluate the Safety and Immunogenicity of a Two-Dose series of Monovalent A/Indonesia/5/05 (H5N1) Vaccine Antigen in Adults Aged ≥ 18 Years"

"A Phase 2, Observer-Blind, Randomized, Multi-Center Trial to Evaluate the Safety and Immunogenicity of Heterologous Booster Vaccination with Monovalent A/Turkey/Turkey/1/05 (H5N1) Vaccine Antigen Adjuvanted with XXX given 6 or 12 Months Following a Single Priming Dose of Monovalent A/Indonesia/5/05 (H5N1) Vaccine Antigen Adjuvanted with XXX in Adults 18 Years of Age or Older"

"Assessment of the Effect of XXX and Naproxen Sodium Combination Tablet, XXX Tablet, and Naproxen Sodium Tablet Treatment on Blood Pressure When Administered Intermittently for Six Months for the Acute Treatment of Migraine Attacks, with or without Aura, in Adults"

"A Phase 2a Randomized, Double Blind, Placebo Controlled Trial to Evaluate the Safety and Immunogenicity of a Seasonal Influenza Virus-Like Particle (VLP) Vaccine (recombinant) in Healthy Adults"

"A Six Week Double-Blind, Randomized, Multicenter Comparison Study of the Analgesic Effectiveness of XXX 200 mg BID Compared to XXX 50 mg QID in Subjects with Chronic Low Back Pain"

"Lot Consistency, Immunogenicity, and Safety Study of Three Lots of XXX Vaccine Administered by Intradermal Route in Comparison with Standard XXX Administered Intramuscularly in Adult Subjects Aged 18 to 64 Years"

"A Phase II, Multicenter, Randomized, Observer-Blind, Placebo-Controlled Study to Evaluate the Immunogenicity, Safety and Tolerability of XXX 2009 H1N1 Influenza Vaccine XXX in Healthy Adults Aged 18 Years and Older"

"A Phase II, Multicenter, Randomized, Observer-blind, Placebo-Controlled Study to Evaluate the Immunogenicity, Safety and Tolerability of XXX 2009 H1N1 Influenza Vaccine XXX in a Healthy Pediatric Population"

"A study to evaluate the safety and immunogenicity of an A/California/7/2009 (H1N1)v-like vaccine XXX or XXX co-administered with trivalent inactivated seasonal influenza vaccine in adults 19 to 40 years of age"

"Immunogenicity and Safety of Multiple Formulations of an A/H1N1 Pandemic Vaccine in Healthy Adult and Elderly Subjects"

"Immunogenicity and Safety of Multiple Formulations of an A/H1N1 Pandemic Vaccine in Healthy Subjects Aged 6 Months to 9 Years"

"Safety and Immunogenicity of Revaccination with Influenza Vaccine in Healthy Adult Subjects aged 18 to 64 Years who were previously vaccinated with XXX or XXX"

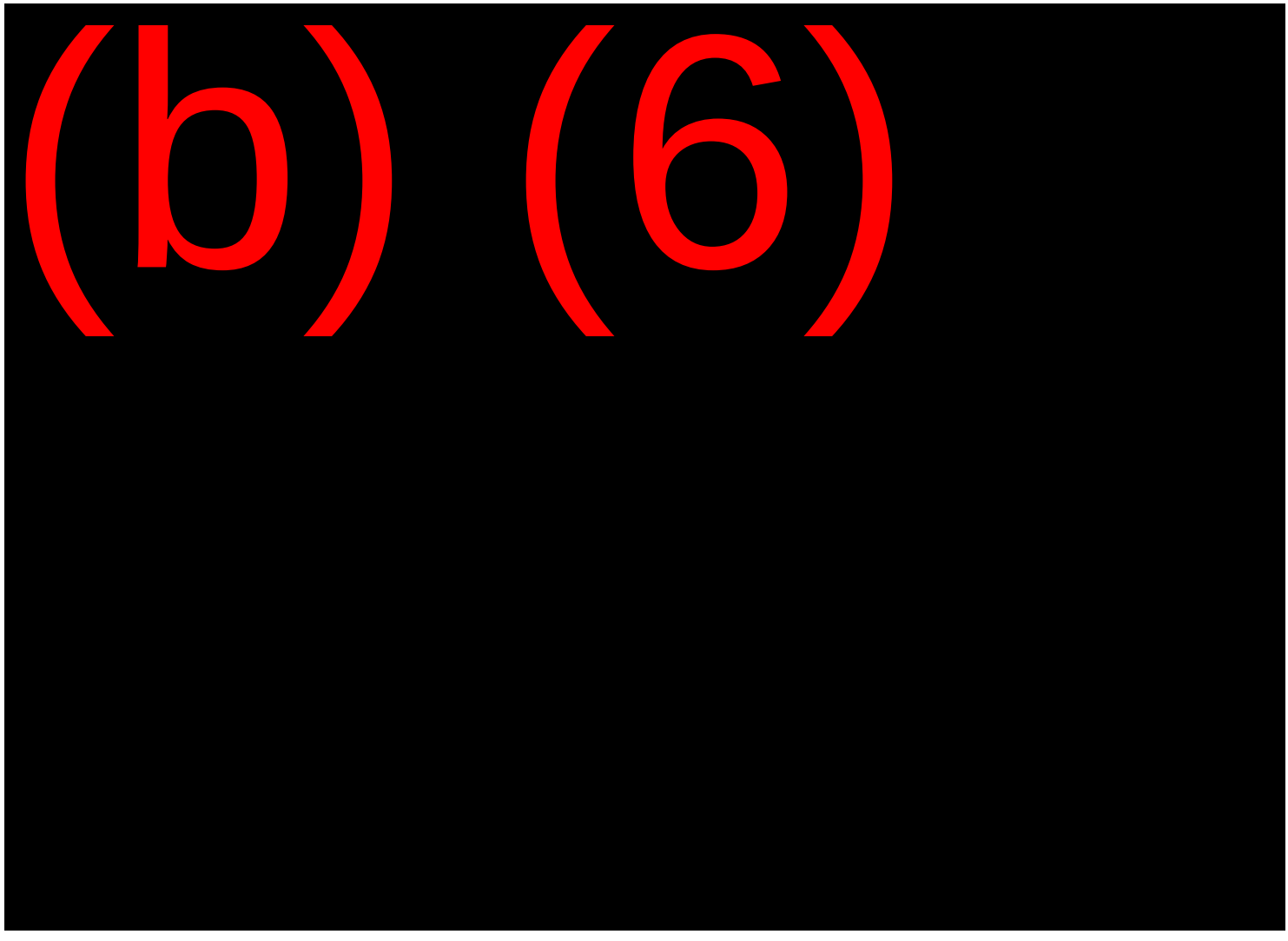
"Randomized, Double-Blind, Placebo-Controlled Study to Assess the Immunogenicity and Safety of a Cell-Derived Trivalent Subunit Influenza Vaccine in Adults Aged ≥18 years and ≤ 64 years During Two Consecutive Years"

"Randomized, double-blinded, active-controlled, multi-center, three-arm, Phase III trial of 4940 subjects in two age strata (6 months to ≤ 35 months and 3 years to < 9 years of age). (Safety and Immunogenicity Among Children Administered Quadrivalent Influenza Vaccine)"

"*An Open Label Study to Evaluate the Contraceptive Efficacy and Safety of XXX XXX Transdermal Delivery System"*

"Immunogenicity and safety study of one dose of XXX meningococcal vaccine XXX (blinded lots) versus one dose of XXX in healthy subjects aged 10-25 years"

Revised 01/07/2011


(b) (6)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

STATEMENT OF INVESTIGATOR

(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)
(See instructions on reverse side.)

Form Approved: OMB No. 0910-0014.
Expiration Date: May 31, 2009.
See OMB Statement on Reverse.

NOTE: No investigator may participate in an investigation until he/she provides the sponsor with a completed, signed Statement of investigator. Form FDA 1572 (21 CFR 312.53(c)).

1 NAME AND ADDRESS OF INVESTIGATOR

Robert J. Jeanfreau, MD
Benchmark Research
3800 Houma Blvd, Suite 345
Metairie, LA 70006

2 EDUCATION, TRAINING, AND EXPERIENCE THAT QUALIFY THE INVESTIGATOR AS AN EXPERT IN THE CLINICAL INVESTIGATION OF THE DRUG FOR THE USE UNDER INVESTIGATION ONE OF THE FOLLOWING IS ATTACHED.

☒ CURRICULUM VITAE ☐ OTHER STATEMENT OF QUALIFICATIONS

3 NAME AND ADDRESS OF ANY MEDICAL SCHOOL, HOSPITAL, OR OTHER RESEARCH FACILITY WHERE THE CLINICAL INVESTIGATION(S) WILL BE CONDUCTED

Benchmark Research
3800 Houma Blvd, Suite 345
Metairie, LA 70006

4 NAME AND ADDRESS OF ANY CLINICAL LABORATORY FACILITIES TO BE USED IN THE STUDY.

Spectra Clinical Research
8 King Road
Rockleigh, NJ 07647

5 NAME AND ADDRESS OF THE INSTITUTIONAL REVIEW BOARD (IRB) THAT IS RESPONSIBLE FOR REVIEW AND APPROVAL OF THE STUDY(IES).

Copernicus Group IRB
1 Triangle Drive, Suite 100
Durham, NC 27713

6 NAMES OF THE SUBINVESTIGATORS *(e.g. research fellows, residents associates)* WHO WILL BE ASSISTING THE INVESTIGATOR IN THE CONDUCT OF THE INVESTIGATION(S).

George H. Bauer, Jr. M.D.
Jeffery J. Segner, MSPH, CCRC
Cori C. Favaloro, CCRC
Donna C. Goodman, CRC
Kristen M. Knight, A.D., CCRC
Jenna D. Griffin, B.S. Assistant CRC
Connie T. Lewis, CRC
Amelia Walch-Patterson, M.P.H., Assistant CRC

7. NAME AND CODE NUMBER, IF ANY, OF THE PROTOCOL(S) IN THE IND FOR THE STUDY(IES) TO BE CONDUCTED BY THE INVESTIGATOR.

A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid X-22-201 (IND# 103,589)

EF

8. ATTACH THE FOLLOWING CLINICAL PROTOCOL INFORMATION:

☐ FOR PHASE 1 INVESTIGATIONS, A GENERAL OUTLINE OF THE PLANNED INVESTIGATION INCLUDING THE ESTIMATED DURATION OF THE STUDY AND THE MAXIMUM NUMBER OF SUBJECTS THAT WILL BE INVOLVED.

☒ FOR PHASE 2 OR 3 INVESTIGATIONS, AN OUTLINE OF THE STUDY PROTOCOL INCLUDING AN APPROXIMATION OF THE NUMBER OF SUBJECTS TO BE TREATED WITH THE DRUG AND THE NUMBER TO BE EMPLOYED AS CONTROLS, IF ANY; THE CLINICAL USES TO BE INVESTIGATED; CHARACTERISTICS OF SUBJECTS BY AGE, SEX, AND CONDITION; THE KIND OF CLINICAL OBSERVATIONS AND LABORATORY TESTS TO BE CONDUCTED; THE ESTIMATED DURATION OF THE STUDY; AND COPIES OR A DESCRIPTION OF CASE REPORT FORMS TO BE USED.

9. COMMITMENTS:

I agree to conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.

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I agree to report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with 21 CFR 312.64.

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I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

I agree to maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.

I will ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312.

INSTRUCTIONS FOR COMPLETING FORM FDA 1572
STATEMENT OF INVESTIGATOR:

1. Complete all sections. Attach a separate page if additional space is needed.
2. Attach curriculum vitae or other statement of qualifications as described in Section 2.
3. Attach protocol outline as described in Section 8.
4. Sign and date below.
5. FORWARD THE COMPLETED FORM AND ATTACHMENTS TO THE SPONSOR. The sponsor will incorporate this information along with other technical data into an Investigational New Drug Application (IND). INVESTIGATORS SHOULD NOT SEND THIS FORM DIRECTLY TO THE FOOD AND DRUG ADMINISTRATION.

10. SIGNATURE OF INVESTIGATOR	11. DATE
(b) (6) [redacted]	4/11/11

(**WARNING:** A willfully false statement is a criminal offense. U.S.C. Title 18, Sec. 1001.)

Public reporting burden for this collection of information is estimated to average 100 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Office of the Chief Information Officer (HFA-710)
5600 Fishers Lane
Rockville, MD 20857

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Please DO NOT RETURN this application to this address.

benchmark research

Robert J. Jeanfreau, M.D.

(b) (6) [redacted]

Benchmark Research
3800 Houma Blvd., Suite 345
Metairie, LA 70006
(504) 888-9493 Phone
(504) 454-6868 Fax

Jeanfreau & Jeanfreau Associates
3800 Houma Blvd., Suite 335
Metairie, LA 70006

Licensure: *Louisiana: #*(b) (6) [redacted]

Medical Education

1982-1985	*University Medical Center* *Intern and Resident in Internal Medicine, Lafayette, LA*
1978 – 1982	*LSU School of Medicine, MD, New Orleans, LA*
1973-1977	*Loyola University, BA Psychology, Magna Cum Laude*

Professional Experience

2006-Present	*Benchmark Research* *Principal Investigator* *Metairie, LA*
1986-Present	*Jeanfreau and Jeanfreau Associates* *Private Practice* *Metairie, LA*

Professional Affiliations

Association of Clinical Research Professional Member, 2006

Drug Information Association, Member, 2006

American College of Physicians
Associate Member, 1983

Southern Medical Association,
Associate Member, 1982

American Medical Association, 1982

Association of Military Surgeons of the U.S.
Member, 1989

Certifications

APPI Physician Investigator Certification, 2008

Federal Licensing Examination, 1982

Board eligible in Internal Medicine, 1985

Board certified in Internal Medicine, 1986

Selected Papers and Publications

2010 Halperin, S., Gupta, A., **Jeanfreau, R.,** Klein, N., Reisinger, K., Walter, E., Bedell, L., Gill, C., Dull, P. Comparison of the safety and immunogenicity of an investigational and a licensed quadrivalent meningococcal conjugate vaccine in children 2-10 years of age. *Vaccine Journal 28.*

2010 Talaat, K., Greenberg, M., Lai, M., Hartel, G., Wichems, C., Rockman, S., **Jeanfreau, R.,** Ghosh, M., Kabongo, M., Gittleson, C., and Karron, R. A Single Dose of Unadjuvanted Novel 2009 H1N1 Vaccine is Immunogenic and Well Tolerated in Young and Elderly Adults. *Chicago Journals – The Journal of Infectious Diseases.*

2010 Baxter, R., Blatter, M., Block, S.L., Bruce L., Dewe, W., Jain, V.K., **Jeanfreau, R.,** Pichichero, M., Innis, A. Phase III Evaluation of Immunogenicity and Safety of Two Trivalent Inactivated Seasonal Influenza Vaccines in US Children. *Pediatric Infectious Disease Journal.*

Selected Clinical Trial Experience

"A Phase III, open-label period followed by a randomized, double-blind, placebo-controlled study of the efficacy and safety of XXX compared to placebo in subjects with Chronic Low Back Pain."

"A Phase III Study of the efficacy, tolerability and safety of XXX in the treatment of pain associate with Osteoarthritis Flare of the Knee, including a randomized double-blind, placebo-controlled, parallel group Phase I followed by an Open-Label treatment Phase."

"A Phase II, double-blind, randomized, placebo-controlled study to assess the effect of XXX for induction of healing of Diabetic Foot Ulcer."

"A Phase III, single-blind, randomized study to evaluate the immunogenicity and safety of XXX compared with XXX administered intramuscularly in children (6 months and older). "

"A clinical study to evaluate the safety and efficacy of XXX v. XXX in the treatment of Allergic Rhinitis."

"A Multi-center, open-label, randomized, study to evaluate depressed patients treated with XXX vs. XXX in a primary care setting."

"A Phase II open-label, single-arm trial to evaluate the shedding and safety of XXX administered to children 6 <60 months of age."

"Double-blind, multi-centre, placebo-controlled, clinical study to evaluate the clinical equivalence of XXX vs. XXX for the indication of Seasonal Allergic Rhinitis"

"A Phase II, Double-Blind, Randomized, Placebo-Controlled, Multicenter, Dose-Ranging Study of 100 or 250 ug XXX to Assess the Efficacy and Safety of the Vaccine as an Aid to Smoking Cessation"

"A Phase II, Prospective, Randomized, Open-Labeled Study to Evaluate3 the Immune Responses of XXX Compared with XXX Vaccine in Children 12 to <36 Months"

"A Combined Phase II/III, Randomized, Multicenter Study to Evaluate Safety, Tolerability and Immunogenicity of XXX Vaccines, or XXX, in Healthy Children and Adolescents"

"Double Blind, Placebo Controlled, Phase III Study of The Efficacy of an Investigational Vero Cell Derived Influenza Vaccine (VCIV) to Prevent Culture Confirmed Influenza Infection (CCII)"

"The Effect of Dose Titration and Dose Tapering On the Tolerability Of XXX in Women With Vasomotor Symptoms Associated With Menopause"

"An Investigator-Blinded Study Evaluating the Efficacy and Safety of Four XXX Treatment Regimens with Placebo and XXX in the Treatment of Onychomycosis of the Toenail"

"A Multi-Center, Double-Blind, Randomized, Parallel Group Study Evaluating the Therapeutic Equivalence and Safety of XXX XXX Pharmaceuticals XXX XXX Vaginal Cream, 2% and XXX Pharmaceutical XXX (XXX) Vaginal Cream, 2% and Both Active Treatments to a Vehicle Control in the Treatment of Vulvovaginal Candidiasis Caused by Candida Species"

"A Randomized, Double Blind, Placebo Controlled, Parallel Group, Multi-Center Study with a Double-Blind Extension Investigating the Efficacy and Safety of a XXX (XXX) Formulation of XXX for the Treatment of Nocturia in Adults"

"A Multi-Center Extension Study Investigating the Long Term Efficacy and Safety of a Fast-Dissolving (XXX) Formulation of XXX for the Treatment of Nocturia in Adults"

"A trial to Evaluate the Safety and Immunogenicity of a Two-Dose Series of XXX (XXX) Vaccine Antigen in Association with XXX XXX in Adults aged Greater that or Equal to 18 Years"

"A Multi-center, Double-Blind, Active-Controlled, Parallel Group Study to Examine The Safety, Tolerability and Efficacy of Oral XXX for the Ling Term Treatment of Acute Migraine with or without Aura"

"A Combined Phase II/III, Observer-Blind, Randomized, Multi-Center Study to Evaluate Safety, Tolerability and Immunogenicity of XXX XXX Vaccines, Produced either in Mammalian Cell Culture of in Embryonated Hen Eggs (XXX), in Healthy Children and Adolescents Aged 3 to 17 Years"

"A Phase II, Prospective, Randomized, Open-Label Study to Evaluate the Immune Responses of XXX Compared with XXX XXX Vaccine (XXX) in Children 12 to <36 Months of Age"

"A Phase II, Double-Blind, Randomized, Placebo-Controlled, Multicenter, Dose-Ranging Study of XXX or XXX of XXX to Assess the Efficacy and Safety of the Vaccine as an Aid to Smoking Cessation"

"Evaluation of the Immunogenicity, Safety, Reactogenicity, Efficacy, Effectiveness and Lot Consistency of XXX XXX-XXX Vaccine in Healthy Adults Aged 18-49"

"Randomized, Double Blind, Placebo-Controlled Study to Assess Whether the Administration of XXX Could Facilitate the Discontinuation of XXX (XXX) Therapy in Subjects with Chronic Insomnia"

"A phase III, observer-blind, multi-centre, multi-country, randomized study to evaluate the immunogenicity and safety of thimerosal-free XXXX compared with XXXX administered intramuscularly in children (6 to 35 months of age)"

"Double Blind, Placebo controlled Phase 3 Study of the Efficacy of an Investigational Vero Cell-Derived Influenza Vaccine to Prevent Culture Confirmed Influenza Infection"

"Phase 2, observer-blind, randomized, multi-center trail to evaluate the safety and immunogenicity of heterologous booster vaccination with monovalent XXXX vaccine antigen adjuvanted with XXXX 6 to 12 months following a single priming dose of monvalent XXXX vaccine antigen adjuvanted with XXXX in adults 18 years of age and older"

"A phase 3, Randomized, Observer-blind, Multi-Center Study to Compare the Safety and Immunogenicity of One-Dose of XXXX Meningococcal Conjugate Vaccine with One Dose of Licensed Meningococcal Conjugate Vaccine XXXX Administered to Healthy Children 2-10 Years of Age"

"A Randomized, Double-blind, Parallel Group Study Evaluating the Efficacy and Safety of Co-administration of a Triple Combination therapy of XXXX, XXXX and XXXX in Subjects with Hypertension"

"A Phase III, observer-blind, randomized, placebo-controlled, multi-center trial to evaluate the safety and immunogenicity of a two-dose series of monovalent XXXX vaccine antigen in association with XXXX adjuvant in adults ≥ 18 years".

"A Phase II, Multi-center, Randomized, Observer-Blind, Placebo-Controlled Study to Evaluate the Immunogenicity, Safety and Tolerability of XXX 2009 H1N1 Influenza Vaccine XXX in Healthy Adults Aged 18 Years and Older."

"A Phase II, Multi-center, Randomized, Observer-blind, Placebo-Controlled Study to Evaluate the Immunogenicity, Safety and Tolerability of XXX 2009 H1N1 Influenza Vaccine XXX in a Healthy Pediatric Population."

"A phase II, randomized, observer blind, controlled, Multi-center study to assess immunogenicity and antibody persistence following vaccination with XXX candidate combined measles, mumps, and rubella vaccine (MMR) versus XXX as a first dose, both administered subcutaneously at 12-15 months of age, concomitantly with hepatitis A vaccine (HAV), varicella vaccine (VV) and pneumococcal conjugate vaccine (PCV) but at separate sites."

"A Randomized, Partially Blind Active Controlled Study to Evaluate the Immunogenicity of XXX in Adults 18 to 49 years of Age"

"Immunogenicity and Safety of Multiple Formulations of an A/H1N1 Pandemic Vaccine in Healthy Subjects Aged 6 Months to 9 Years"

"Multi-Year Efficacy Study of XXX High-Dose Trivalent Vaccine Compared with XXX Vaccine in Adults ≥ 65 Years of Age"

"Self-Selection and Self-Recognition Study for Over-the-Counter (OTC) XXX XXX 0.4mg capsules"

"Immunogenicity and safety study of XXX influenza vaccine (XXX) when administered in children"

"A Multi-Center, Randomized, Controlled Clinical Trial to Evaluate the Safety and Effectiveness of XXX Dermal Regeneration Template for the Treatment of Neuropathic Diabetic Foot Ulcers"

"A Randomized, Double-blind, Active Controlled Study to Evaluate the Immunogenicity of XXX in Children 2 to 17 Years of Age"

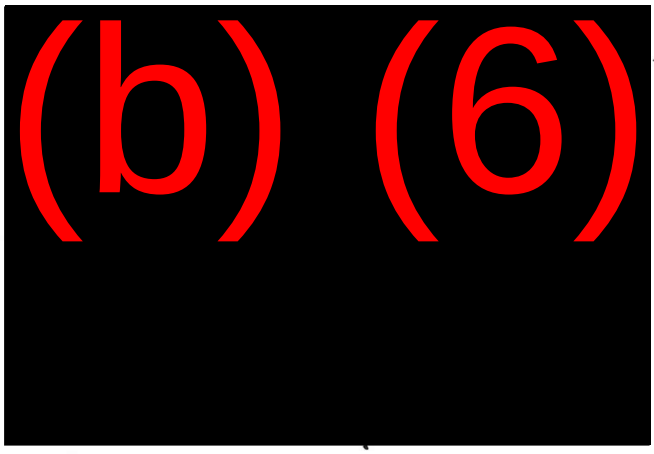
"A Second Phase 3, Multi-Center, Randomized, Double-Blind, Placebo-Controlled Study to Assess Efficacy, Immunogenicity and Safety of XXX A Conjugate Vaccine (XXX) as an Aid to Smoking Cessation"

"Health-Related Quality-of-Life and Health-Care Resource Utilization Assessment in XXX and XXX Phase 3 Studies"

"A Phase 3 Randomized, Double-Blind, Placebo-Controlled, Multicenter Study of the Analgesic Efficacy and Safety of the Subcutaneous Administration of XXX in Patients with Osteoarthritis of the Knee"

"Randomized, double-blinded, active-controlled, multi-center, three-arm, Phase III trial of 4940 subjects in two age strata (6 months to ≤ 35 months and 3 years to < 9 years of age)"

Revised: 18Jan2011


(b) (6)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

STATEMENT OF INVESTIGATOR

(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)
(See instructions on reverse side.)

Form Approved: OMB No. 0910-0014.
Expiration Date: May 31, 2009.
See OMB Statement on Reverse.

NOTE: No investigator may participate in an investigation until he/she provides the sponsor with a completed, signed Statement of Investigator, Form FDA 1572 (21 CFR 312.53(c)).

1. NAME AND ADDRESS OF INVESTIGATOR

Ivan H. Rarick II, M.D.
Benchmark Research
3701 J Street, Suite 220
Sacramento, CA 95816

2. EDUCATION, TRAINING, AND EXPERIENCE THAT QUALIFY THE INVESTIGATOR AS AN EXPERT IN THE CLINICAL INVESTIGATION OF THE DRUG FOR THE USE UNDER INVESTIGATION. ONE OF THE FOLLOWING IS ATTACHED.

☒ CURRICULUM VITAE ☐ OTHER STATEMENT OF QUALIFICATIONS

3. NAME AND ADDRESS OF ANY MEDICAL SCHOOL, HOSPITAL, OR OTHER RESEARCH FACILITY WHERE THE CLINICAL INVESTIGATION(S) WILL BE CONDUCTED.

Benchmark Research
3701 J Street, Suite 220
Sacramento, CA 95816

4. NAME AND ADDRESS OF ANY CLINICAL LABORATORY FACILITIES TO BE USED IN THE STUDY.

Spectra Clinical Research
8 King Road
Rockleigh, NJ 07647

5. NAME AND ADDRESS OF THE INSTITUTIONAL REVIEW BOARD (IRB) THAT IS RESPONSIBLE FOR REVIEW AND APPROVAL OF THE STUDY(IES).

Copernicus Group IRB
1 Triangle Drive, Suite 100
Durham, NC 27713

6. NAMES OF THE SUBINVESTIGATORS *(e.g., research fellows, residents, associates)* WHO WILL BE ASSISTING THE INVESTIGATOR IN THE CONDUCT OF THE INVESTIGATION(S).

Thomas F. Gardner, D.O.
Linda Honda, FNP
Nav Nagra-Kooner, CCRC
Eric Garcia, CCRC
Jessica A. Sullivan
Lisa Hadley

7. NAME AND CODE NUMBER, IF ANY, OF THE PROTOCOL(S) IN THE IND FOR THE STUDY(IES) TO BE CONDUCTED BY THE INVESTIGATOR.

A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid X-22-201 (IND# 103,589)

PSC Graphics (301) 443-6740 EF

8. ATTACH THE FOLLOWING CLINICAL PROTOCOL INFORMATION:

☐ FOR PHASE 1 INVESTIGATIONS, A GENERAL OUTLINE OF THE PLANNED INVESTIGATION INCLUDING THE ESTIMATED DURATION OF THE STUDY AND THE MAXIMUM NUMBER OF SUBJECTS THAT WILL BE INVOLVED.

☒ FOR PHASE 2 OR 3 INVESTIGATIONS, AN OUTLINE OF THE STUDY PROTOCOL INCLUDING AN APPROXIMATION OF THE NUMBER OF SUBJECTS TO BE TREATED WITH THE DRUG AND THE NUMBER TO BE EMPLOYED AS CONTROLS, IF ANY; THE CLINICAL USES TO BE INVESTIGATED; CHARACTERISTICS OF SUBJECTS BY AGE, SEX, AND CONDITION; THE KIND OF CLINICAL OBSERVATIONS AND LABORATORY TESTS TO BE CONDUCTED; THE ESTIMATED DURATION OF THE STUDY; AND COPIES OR A DESCRIPTION OF CASE REPORT FORMS TO BE USED.

9. COMMITMENTS:

I agree to conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.

I agree to personally conduct or supervise the described investigation(s).

I agree to inform any patients, or any persons used as controls, that the drugs are being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

I agree to report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with 21 CFR 312.64.

I have read and understand the information in the investigator's brochure, including the potential risks and side effects of the drug.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

I agree to maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.

I will ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312.

INSTRUCTIONS FOR COMPLETING FORM FDA 1572
STATEMENT OF INVESTIGATOR:

1. Complete all sections. Attach a separate page if additional space is needed.
2. Attach curriculum vitae or other statement of qualifications as described in Section 2.
3. Attach protocol outline as described in Section 8.
4. Sign and date below.
5. FORWARD THE COMPLETED FORM AND ATTACHMENTS TO THE SPONSOR. The sponsor will incorporate this information along with other technical data into an Investigational New Drug Application (IND). INVESTIGATORS SHOULD NOT SEND THIS FORM DIRECTLY TO THE FOOD AND DRUG ADMINISTRATION.

10. SIGNATURE	11. DATE
(b) (6)	08/April/2011

(**WARNING:** [redacted] 8, Sec. 1001.)

Public reporting burden for this collection of information is estimated to average 100 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Office of the Chief Information Officer (HFA-710)
5600 Fishers Lane
Rockville, MD 20857

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Please DO NOT RETURN this application to this address.

(b) (6) [redacted]

benchmark research

Ivan H. Rarick II, M.D.

Benchmark Research
3701 J Street, Suite 220
Sacramento, CA 95816, USA
(916) 452-5830 Office
(916) 452-5879 Fax

Licensure: California (b) (6)

Certification: The Medical Board of California
Certified – 1961, Current through 4/30/2011

Medical Education

1963-1965	Sonoma County Hospital Family Practice Residency Santa Rosa, CA
1961-1963	U.S. Navy Solo Medical Officer USS Bexar; Naval Training Center San Diego, CA
1960-1961	Detroit Receiving Hospital Family Practice Internship
1957-1960	University of California M.D. Degree San Francisco, CA
1953-1957	University of California-Berkeley B.A. Degree Phi Beta Kappa

Professional Experience

2006-Present	Benchmark Research Principal Investigator Sacramento, CA
1999– 2007	Physician Provider Midtown Medical Center Sacramento, CA

1970-1999	Private Practice Travis-Rarick Medical Group Sacramento, CA

Certifications & Affiliations

Diplomat of American Board of Family Practices, 1965

Recertified 1999 to 2013

Mercy American River/San Juan Hospital, Active Staff 1965-1999

Sutter Roveville Hospital Active Staff 1994-1999

U.C. Davis School of Medicine Clinical Professor 1974-1997

Mercy American River Hospital "IPC CTTE" 1981-1991

Sacramento Physicians Network Board of Directors 1989-1992

Blue Shield HMO Quality Assurance/ Peer Review Ctte 1991-1995

Greater Sacramento Surgery Center QA/UR Ctte 1993-1999

Diplomate of American Academy of Family Practice Lifetime Member

California Academy of Family Practice member

Sacramento Academy of Family Practice member

California Medical Association member

Sierra-Sacramento Valley Medical Society member, Spirit Program Volunteer Physician County Primary Care Clinic

Professional Conduct & Ethics Ctte member

Volunteer Physician Flying Samaritans Medical Mission

San Quintin Clinic Baja California sur Mexico

Selected Clinical Trial Experience

" A randomized, double-blind, placebo controlled trial to evaluate the efficacy of XXX in healthy adults 18-49 years of age"

"A study to assess the efficacy of XXX vs. XXX in relief of provoked craving."

"A 24-Week, Blinded, Randomized, Placebo-Controlled Dose-Ranging Trial of XXX for Weight Loss in Healthy Obese Patients"

"A Phase III, Open-Lable, Long-Term Safety Study of XXX in the treatment of Moderate to Moderately Severe Pain Associated with Osteoarthritis"

"A Phase II, Double-Blind, Randomized, Placebo-Controlled, Multi, Dose-Ranging Study of XXX to Assess the Efficacy and Safety of the Vaccine as an AID to Smoking Cessation"

"Double-Blind, Placebo-Controlled Phase III Study of the Efficacy of an Investigational XXX Influenza Vaccine to Prevent Culture Confirmed Influenza Infection"

"Evaluation of the Immunogenicty, Safety, Reactogenicity, Efficacy, Effectiveness and Lot Consi of XXX in Healthy Adults Aged 18 to 49"

" Double-Blind, Multi-Center, Randomized, Parallel-Group, Year Long Study to Assess the Efficacy and Safety of XXX Administered Orally Once Daily with an Initial 6-Week Low Calorie Diet in Obese Males and Females"

"A Multi-Center, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study Evaluating the Efficacy and Impact on Health-Related Quality of Life of XXX 5 mg Once Daily Given for 2 Weeks in Subjects 18 yr of Age and Older with Seasonal Allergic Rhinitis"

"Open-Label Extension (OLE) Safety and Efficacy Study of XXX Following the Year-Long Controlled Clinical Trials of XXX in Obese Males and Females"

"A Phase I/II, Randomized, Observer-blind, Multicenter, Dose Ranging Study

to Evaluate the Immunogenicity, Safety and Tolerability of Different Formulations of an Adjuvanted or Non-Adjuvanted Cell Culture-Derived H5N1 Subunit Influenza Virus Vaccine in Healthy Subjects 18 – 40 Years of Age"

"A Phase III, Observer-Blind, Multi-Centre, Multi-Country, Randomized Study to Evaluate the Immunogenicity and Safety of Thimerosol-Free (TF) XXX Compared with XXX Administered Intramuscularly in Children (6 to 35 months of Age)"

"Double-Blind, Placebo Controlled Phase III Study of the Efficacy of an Investigational Vero Cell-Derived Influenza Vaccine (VCIV) to Prevent Culture Confirmed Influenza Infection (CCII)"

"Phase 3 Double Blind Clinical Study of Effectiveness and Safety of Vero Cell-Derived, Trivalent, Seasonal Influenza Vaccine (VCIV) in Adults 50 Years of Age and Older"

"A Phase III, Randomized, Double-Blind, Parallel Group, Placebo Controlled, Multicenter Study to Assess the Efficacy and Safety of the Beta-3 Agonist XXX in Subjects with Symptoms of Overactive Bladder"

"A Randomized, Double-Blind, Parallel Group, Active Controlled, Multi- center Long-term Study to Assess the Safety and Efficacy of the Beta-3 Agonist XXX (50 mg qd and 100 mg qd) in Subjects with Symptoms of Overactive Bladder"

"A Randomized, Partially Blind Active Controlled Study to Evaluate the Immunogenicity of XXXX in Adults 18 to 49 years of Age."

"A Randomized, Double-Blind, Active Controlled Study to Evaluate the Immunogenicity of XXXX in Adults 18 to 49 Years of Age."

"A Randomized, Double-blind, Placebo-controlled Study to Evaluate the Safety of XXXX in Children."

"A Phase 2, Randomized, Double-Blind, Vehicle-Controlled, Parallel Group Clinical Study to Evaluate the Safety and Efficacy of XXX Gel in the Symptomatic Treatment of Osteoarthritis of the Knee"

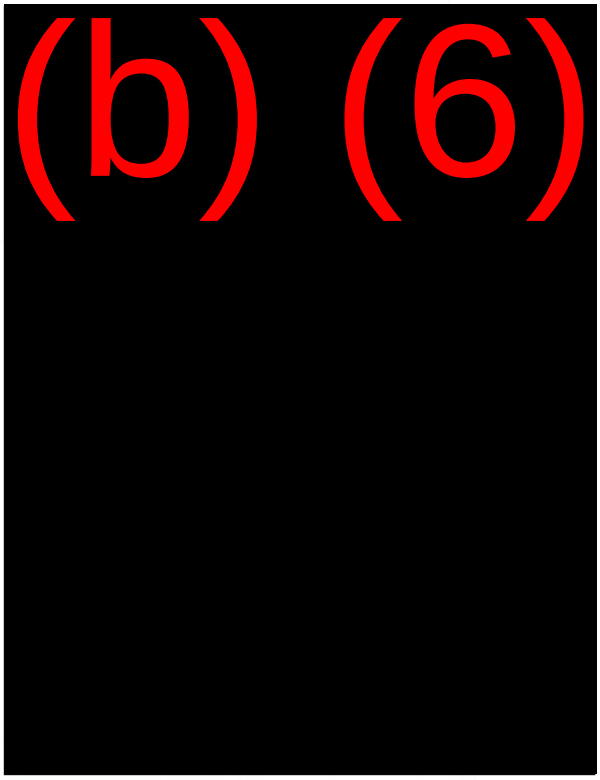
"Immunogenicity and safety study of XXX influenza vaccine (XXX) when administered in children."

"Immunogenicity and safety study of one dose of XXX meningococcal vaccine XXX (blinded lots) versus one dose of XXX in healthy subjects aged 10-25 years"

"A Randomized, Double-blind, Active Controlled Study to Evaluate the Immunogenicity of XXX in Children 2 to 17 Years of Age"

"A Phase 3 Randomized, Double-Blind, Placebo-Controlled, Multicenter Study of the Analgesic Efficacy and Safety of the Subcutaneous Administration of XXX in Patients with Osteoarthritis of the Knee"

Revised 01.07.11


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